

*Blood Diseases of
Infancy and Childhood*

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To my wife
MARGARET

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Preface

The purpose of this book is to present the essentials of pediatric hematology in concise form for the medical student and practitioner. In lectures over a period of many years to members of these groups I have been impressed with the need for providing a textbook in which the salient features of blood dyscrasias are presented against the background of normal development of infancy and childhood.

Although these hematologic disorders correspond closely to those occurring in adult life the approach to diagnosis is frequently complicated by concomitant alterations which normally take place during growth within and outside of the hematopoietic system. Certain of the blood disorders occur almost exclusively in early life and are best described in a pediatric setting.

The rapid development of pediatric hematology can be strikingly illustrated by recalling the fate of von Jaksch's anemia. This syndrome which dominated the discussions of anemia in pediatric textbooks thirty years ago receives scant mention or is entirely omitted from present day publications. The dismemberment of this heterogeneous group of blood disorders replaced by more soundly based conditions reflects the evolution of new concepts in a rapidly expanding field of medicine. Similarly, such diverse conditions as thrombocytopenic purpura, erythroblastosis, and the hemolytic anemias and disorders of the coagulation mechanism which occur so prominently in younger persons have been clarified by recent contributions from the growing field of immunohematology and by the identification of multiple factors responsible for blood clotting. In the hereditary hemolytic syndromes important information has been acquired by the application of genetic analysis and the characterization of abnormal types of hemoglobin by electrophoretic examination. The discovery that drug sensitivity and fialism are based on a genetically transmitted enzyme deficiency suggests that other unexplained hematologic entities may also eventually be included in the growing list of inborn errors of metabolism.

The search for a fetal etiology of congenital anomalies has also had its impact on the blood dyscrasias in the pediatric age group. This orientation has been so attractive that the pathogenesis and interpretation of the blood disorders especially in the early months and years of life require an examination of the maternal-fetal relationships and of environmental and genetic influences and a consideration of normal embryologic development and its aberrations.

Although the blood diseases of younger patients are effectively presented in the comprehensive hematologic textbooks currently available nevertheless there is much to be gained by their separate consideration. This book is not intended however to supplant these larger works rather it is designed to serve as a companion volume since few diseases in this or other specialties can be arbitrarily restricted to a specific age period. It is planned throughout to emphasize the pathogenesis of hematologic disorders of the pediatric age group in the light of established concepts as a basis for rational treatment.

From the rapidly enlarging mass of hematologic and pediatric contributions only that segment is represented which bears pertinently on the interpretation, diagnosis and management of the blood diseases encountered in pediatric practice.

Elaborate techniques have been developed within recent years permitting visualization of physiologic forces controlling blood formation and their abnormalities. Although certain important data can be acquired with these facilities I have always felt that the detection of the common blood disorders lies within the province of every medical practitioner by careful history and physical examination with the use of simple instruments and techniques and by coordinating information derived from a variety of interrelated sources. In striving to achieve this objective, it is hoped that an easily understandable and practical book has been prepared.

I wish to acknowledge my indebtedness to the authors of the standard hematologic, pathologic and pediatric textbooks which were of inestimable value for orientation and subject matter. Thanks are due to my colleagues and associates who offered suggestions in various aspects of the text many of whom are identified in papers written conjointly and are mentioned in the bibliographies. I wish particularly to express my appreciation to those who reviewed one or more chapters of the book and whose advice and criticism were invaluable notably Dr Marion E. Erlandson, Dr Sydney S. Gellis, Miss Jane Haber, Dr S. Frederick Rabiner and Dr Marjorie B. Zucker. Above all I am everlastingly indebted to my wife, Margaret, for supervising and contributing to every phase of the preparation of the manuscript thereby relieving me of numerous tasks that disturb the equanimity needed for writing. For added participation of my family, thanks are due my daughter, Christine, for help in revising the manuscript and my son, Carl, for emphasizing the point of view and needs of the medical student. My deep appreciation is extended to Dr Samuel Z. Levine, Professor of Pediatrics of the Cornell University Medical College, who by his constant support and warm friendship provided me with the needed encouragement and stimulation to develop the field of pediatric hematology in his department and the material on which this book is based. Thanks are due Mr. Percy Brooks and his associates of the Department of Illustration of The New York Hospital-Cornell Medical Center for cooperation in the preparation of the figures. Sincere appreciation is due Mrs. Pindora Munning for her patience in typing and retyping large sections of the manuscript.

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Origin and Development of Blood Cells

Blood Formation in the Fetus A review of the essential features of prenatal blood development provides a basis for interpreting postnatal abnormalities of the circulating blood elements their progenitors and sites of formation. The designation of prenatal hematopoiesis imports an implication of continuity to the events occurring in the embryo in the first two months and in the fetus in the remainder of gestation. Current emphasis on maternal fetal relationships which has shed so much light on other systems conceivably may clarify the etiology of certain of the blood dyscrasias on the same basis. An understanding of the blood changes in the earlier months of life furthermore requires some knowledge of embryonic and fetal blood formation.

Sites of Blood Formation. Blood cells in the embryo arise from the mesenchyme. The first cells produced in the yolk sac become the primitive red corpuscles. Since the mesenchyme is widespread throughout the embryo, blood formation begins in multiple sites but eventually becomes specialized in certain organs. With some overlapping, definitive blood centers mainly involving red cell elements appear successively in the yolk sac at the fourth month of gestation.² Although the bone marrow makes its appearance in the sixth week of development, it does not become a site of active hematopoiesis until the fourth to fifth month and does not become the exclusive site³ until two to three weeks after birth.⁴ Until the middle of fetal life the liver is the most actively engaged organ participating in blood formation. It has been stated that the liver as opposed to the marrow represents the principal source of fetal erythrocytes. In this period of hepatic hematopoiesis, fetal hemoglobin is the sole type synthesized. As hematopoiesis wanes in this area, it is assumed by the bone marrow, which exercises this function for the remainder of fetal life. Coincidental activity goes on in the spleen, lymph nodes, and to a lesser extent in the thymus.

The bone marrow and spleen provide an ideal environment for red cell and hemoglobin formation. In both there are nonanastomosing arterial capillaries emptying into a rich plexus of venous sinusoids. By virtue of sluggish circulation and blood stasis, a relatively high carbon dioxide tension develops, a factor of

lation possesses completely differentiated precursors. Myeloblasts are precursors of myelocytes and granulocytes. Lymphoblasts of lymphocytes and monoblasts of monocytes. Reticulocytes and mature erythrocytes are derived from pronormoblasts and the more mature normoblasts. Erythroblasts refer either to megakaryoblasts or pronormoblasts. The former occur in patients with pernicious anemia. The latter are the progenitors of the normal red cell series. The megakaryoblast and promegakaryocyte are the primitive forms of the mature megakaryocyte.

Fetal Hemoglobin. The earliest evidence of a difference between fetal and maternal hemoglobin was the greater resistance to alkaline denaturation observed in fetal hemoglobin. Fetal hemoglobin constitutes about 45 to 90 per cent of the hemoglobin of the infant at birth and is rapidly replaced within the first year by adult hemoglobin. Normally, about 15 per cent fetal hemoglobin persists to the age of 1 year, 5 per cent to 2 years, and less than 2 per cent after the age of 4 years.⁶ It is rarely demonstrable thirty months after birth. Occasionally a child continues to have small quantities of alkali resistant hemoglobin as late as 4 years of age.⁶

Cook and co-workers have shown that in infants born after more than 34 weeks gestation there is an inverse relation between gestational age and percentage of fetal hemoglobin. Most infants born after less than 36 weeks gestation had more than 90 per cent fetal hemoglobin at birth, and those born after 36 weeks usually had less than 90 per cent at birth. After 34 weeks gestation the percentage of fetal hemoglobin drops approximately to 3 or 4 per cent per week prenatally, which is similar to the postnatal weekly decrease.

Fetal Hemoglobin Content as an Index of Maturity. The content of fetal hemoglobin has been advanced as an approximate index of maturity, especially in the overmature newborn infant. Cottom⁸ observed a close correlation between the percentage of fetal hemoglobin and the period of gestation but not with the birth weight. The mean fetal hemoglobin ranged from 90 per cent at 35 weeks gestation to values below 60 per cent at 42 weeks. A similar observation was made by Abrahamov and associates¹ who found an average fetal hemoglobin concentration of 85.5 per cent (range 64.1 to 95 per cent) in the normal control group as compared with an average of 65.1 per cent in the postmature newborn infant (range 54.4 to 76.4 per cent).

Fetal Blood and Oxygen Dissociation. Fetal blood takes up oxygen at a tension at which it is relinquished by maternal blood, a mechanism of distinct advantage to the fetus. Eastman and associates¹¹ found that at gas tensions between 25 and 60 mm Hg fetal blood absorbs oxygen more effectively, and that at all gas tensions it releases carbon dioxide more readily than does the blood of the mother. The dissociation curve of fetal blood thus is displaced to the left as compared with that of the pregnant and nonpregnant woman, a circumstance favorable to the uptake of oxygen by the fetus in utero.¹² Barcroft¹³ suggested that the greater affinity for oxygen of fetal blood than maternal blood depends upon the fetal type of hemoglobin, a type which was thought to acquire oxygen readily and shed it with difficulty.

Although it is true that the fetal type of hemoglobin differs chemically from normal adult hemoglobin (see discussion of fetal hemoglobin), evidence that it

primary importance in the elaboration of hemoglobin and in the formation of the primordial cell

Appearance of Blood Elements The mesenchyme is regarded as the essential blood forming tissue of the embryo corresponding to fixed connective tissue cells in the adult organism. The hemocytoblast a derivative of the mesenchyme is the primitive totipotent cell whose main function is involved in hematopoiesis. This early cell frequently umboid in embryos represents the precursor of red blood cells granular leukocytes lymphocytes and megakaryocytes^{10,1}

ERYTHROCYTES In each area of blood formation the erythroblasts multiply rapidly by mitosis and as they mature manifest in sequence the generation of hemoglobin condensation and subsequent loss of the nucleus and final entrance into the circulation. In the first six weeks of gestation practically all red cells are nucleated. A rapid change to non nucleated forms occurs during the ninth week and is practically complete by the end of the tenth week.

The very large primitive erythroblasts representing the first or provisional generation of red cells to appear in the embryo are replaced by the smaller and more numerous erythroblasts formed in the hepatic period. By the end of the fourth month the primitive cells have entirely disappeared.

GRANULOCYTES AND LYMPHOCYTES Granulocytes noted initially in the yolk sac and liver are found in increasing numbers in the fourth month. In the spleen red cell formation is extremely active initially but by the fifth month of gestation it gives way to the formation of lymphocytes.⁴ In another study¹⁵ it was found that red cells alone were in evidence in the blood for the first two and one half months of intrauterine life. At the end of this time granulocytes made their appearance with the lymphocytes appearing at the beginning of the fourth month.

MONOCYTES Although it is still debatable the origin of monocytes has been relegated to the reticular cells of the reticuloendothelial tissue. From these cells in the liver lymph nodes spleen and marrow the progenitor of the monocytes the monoblast is derived.

PLATELETS Megakaryocytes are noted in the yolk sac and liver however with intensive production of erythroblasts in the liver granulocytes and to a lesser extent megakaryocytes are formed. The latter increase in number as the hepatic period of blood formation becomes more vigorous but platelet proliferation from megakaryocytes is not active until the bone marrow period. Although platelets appear in the embryo at the same time as megakaryocytes and are derived from them the origin of the megakaryocytes has been variously traced to hemocytoblasts reticuloendothelium or fused histiocytic elements. In extrauterine life evidence has been cited of active platelet formation from megakaryocytes in the lungs as well as the bone marrow.¹⁴

Theories of Blood Formation There are two theories as to the origin of blood formation. The monophyletic theory regards the hemocytoblast as the totipotent cell from which all types of blood elements both red and white originate. According to the polyphyletic theory each of the blood cell elements in the peripheral circulation possesses completely differentiated precursors. According to the polyphyletic theory each of the blood cell elements in the peripheral circu

lation possesses completely differentiated precursors. Myeloblasts are precursors of myelocytes and granulocytes, lymphoblasts of lymphocytes and monoblasts of monocytes. Reticulocytes and mature erythrocytes are derived from pronormoblasts and the more mature normoblasts. Erythroblasts refer either to megakaryoblasts or pronormoblasts. The former occur in patients with pernicious anemia. The latter are the progenitors of the normal red cell series. The megakaryoblast and promegakaryocyte are the primitive forms of the mature megakaryocyte.

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Although it is true that the fetal type of hemoglobin differs chemically from normal adult hemoglobin (see discussion of fetal hemoglobin), evidence that it

is responsible for the fetal type of oxygen dissociation is inconclusive. Prepared hemoglobin solutions from fetal and maternal blood possess similar affinities for oxygen. It has not yet been determined whether fetal hemoglobin residing within the intact erythrocytes absorbs oxygen at low concentrations more effectively than normal blood. The properties ascribed to fetal blood possibly may depend upon factors inherent in the intact red cell in which fetal hemoglobin plays an influential part.

On the other hand the differences in oxygen uptake in the fetal as compared with the adult red cells may be due to properties in the red cells or plasma exclusive of fetal hemoglobin. It is also conceivable that differences in thickness or thinness of the maternal and fetal red cells may account for the peculiarities of oxygen dissociation and uptake in fetal life. On this basis the maternal red cells which are thinner than those of the fetus⁶ would promote oxygen transfer to the fetus³ where it would be retained because of the greater red cell thickness.

Relation of Cord Blood Hemoglobin to Fetal Oxygen Saturation. The mean umbilical cord hemoglobin obtained by most observers ranges between 15.7 and 17.9 gm per 100 ml of blood.¹⁷ The wide spread of hemoglobin values observed by Marks and co-workers¹ extending from 12 to 22 gm per 100 ml of blood (95 per cent range of 13.7 to 20.1 gm) with a mean value of 16.9 gm is representative of values noted in most series. Marks and colleagues¹⁷ also showed that whether an infant is born with a cord hemoglobin as low as 13 gm or as high as 20 gm the hemoglobin will be established at a level of about 11 gm at 2 months of age.

Studies of the cord blood from infants of varying age of fetal maturity have been analyzed from the standpoint of influences acting on the fetus before birth. Several factors particularly have been investigated: the state of oxygen saturation, the hemoglobin concentration, and a possible relationship between the two. Walker and Turnbull⁴ found that the hemoglobin level during gestation rose steadily from 11 gm per 100 ml in the tenth week to 14 or 15 gm by the twenty-second to the twenty-fourth week. By the thirty-eighth week the mean value was 15.2 gm and by the fortieth week it was 16.5 gm. When pregnancy was prolonged this rise continued. By the forty-third week the mean value was 18.8 gm. They interpreted the increasing hemoglobin value as a response to a falling oxygen supply in the fetus and furthermore decided that postmaturity was associated with abnormally high hemoglobin levels.

Marks and associates¹ and Rooth and Sjostedt³ could find no correlation between cord blood hemoglobin and fetal gestation time. The latter were unable to confirm Walker and Turnbull's⁴ findings that there is a continuous decrease in oxygen saturation with advancing pregnancy. They found a constant mean hemoglobin level of 17 gm per 100 ml from the fortieth to the forty-third week and during these four weeks there was no correlation between cord blood hemoglobin and the duration of pregnancy. On the other hand McKay¹⁶ observed the gradual reduction in the oxygen levels of the cord blood in normal pregnancy as term is approached and passed but no relation to hemoglobin levels.

Cook and co-workers⁷ also showed that intrauterine hypoxia in the postmature

infant was not associated with an increase in percentage of fetal hemoglobin. On the basis of reticulocyte counts and clinical examination they suggested that increased total concentrations of hemoglobin observed in the premature infant with prenatal hypoxia are possibly the result of hemoconcentration rather than an erythropoietic response to lack of oxygen.

Despite these conflicting observations it may be stated that oxygen saturation in the fetus is decreased but that additional data are required with techniques designed to obtain precise intruterine fetal measurements. At present additional causes for the variation in cord hemoglobin levels at birth other than the factor of decreased oxygen saturation alone must be sought.

Survival Time of Fetal Red Cells It is of interest that the life span of fetal red cells when measured by tagging umbilical cord blood at birth with radioactive chromium¹³ is shorter than that of normal adults. Recent studies indicate however that this difference in survival rates of the adult and of the newborn red cells is determined by Cr^{51} is only apparent since elution of the isotope from cord blood hemoglobin after dialysis is considerably greater than that from adult hemoglobin. The method of a differential agglutination also demonstrates that the survival of red cells from infants is only slightly less than that from adults.¹⁰ By using Cr^{51} tagged erythrocytes and autotransfusion or heterotransfusion methods red cell survival of premature and full term infants was also found at normal adult levels during the first week of life but was reduced at the second and third months.¹¹

Fetal Myoglobin Human myoglobin may also be separated into fetal and adult types. Fetal human myoglobin (MbF) exhibits a distinct spectroscopic and electrophoretic behavior which is different from adult muscle pigment (MbA). The muscles of premature and normal newborn infants contain only MbF which is gradually replaced by MbA within the first six months of life comparable in sequence to the period of disappearance of fetal red cell hemoglobin from the blood stream.¹ The purpose and function of both the fetal hemoglobins and myoglobins have not been definitely established.

Bone Marrow at Birth, Infancy and Childhood At birth the bone marrow assumes the role of the dominant hematopoietic organ but the potencies of mesenchymal cells or of their reticular derivatives in the connective tissues for blood formation persist throughout life. In older infants and children in whom the usual blood sites are stressed as in those with the hereditary hemolytic diseases and leukemia compensatory hematopoiesis occurs quite readily in the liver and in the spleen.

So active is the demand for blood cell formation that in the infant and young child all the bones are filled with red marrow. Fat appears in the long bones at about 5 to 7 years of age although incipient changes may occur during infancy. From that time until about the age of 20 years a gradual retrogression occurs so that active marrow is eventually restricted to the trunk (ribs, sternum and vertebrae) and proximal portions of the femur and humerus. Red marrow is also found in the clavicles, scapulae, skull and pelvis.⁹ With the appearance of non-functioning yellow marrow a potential reservoir is created for active blood formation in periods of stress.

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Blood Changes During Growth— Postnatal Period, Infancy, and Childhood

Knowledge of the normal blood values in the growing period is a prerequisite to the interpretation of a particular blood response in infancy and childhood. For this reason the normal changes in hematologic values for each of the developmental periods are included in this discussion. It is recognized that the values to be cited are subject to wide variations both in each individual and among members of an age group. These values are given as yardsticks based on experience and in terms of which abnormalities of graded severity may be judged and are in general agreement with the data available in standard texts and articles dealing with this subject.^{7,8,10,11,12,13,14}

Blood Changes in the Newborn Infant During the first week of life the decline in hemoglobin and red cells in the peripheral blood is at a minimum. Following this initial stationary period a definite decline sets in. The postnatal adjustment is characterized by a normal or slightly increased rate of blood destruction accompanied by diminished or stationary hematopoietic activity. During this period the drop in red cells occurs within the limits of normal survival—namely, 100 to 120 days or approximately 0.8 per cent per day. The changes are usually orderly and gradual and appear to be directed toward the establishment of hematopoietic equilibrium designed to function at a lower level than exists at birth. Although hemolysis accounts for the fall in hemoglobin and red cells the continued drop represents a diminished rate of compensatory regeneration. The fall in hemoglobin represents a gradual adjustment to the increased oxygen saturation of the blood which prevails when the lung replaces the placenta as a source of oxygen. Gardner and co-workers¹ believe that erythrogenesis ceases when the oxygen saturation at birth is elevated from about 65 per cent in the umbilical vein to 95 per cent a few hours after birth. Accordingly red cell formation is at a minimum until the hemoglobin level reaches 11 to 12 gm per 100 ml when regeneration sets in. This regulation by the arterial oxyhemoglobin level implies that during the neonatal period decreases below 11 to 12 gm per 100 ml induce hematopoietic activity whereas elevations above 11 to 12 gm tend to decrease bone marrow activity. Increased reticulocyte formation can be

observed in the anemic infant in the first days of life presumably from blood loss during delivery or the seepage of blood into the maternal circulation

Physiologic Anemia of the Newborn Infant Physiologic anemia of the newborn infant occurs in the first two or three months of life when the red cells and hemoglobin drop concurrently and in equal degrees. This decline proceeds in an orderly fashion and is not accelerated by hyperhemolysis unless pathologic factors set in. Following this neonatal adjustment hemoglobin reaches its lowest level at 11 weeks to 2 months of age and remains fairly constant for the remainder of infancy. During the first two years of life the hemoglobin usually ranges from approximately 10 to 12 gm per 100 ml of blood.

Bone Marrow Changes During the first week of life when the decline in hemoglobin and red cells is at a minimum a marked drop in nucleated red cells occurs in the bone marrow. The lowest levels are reached between the end of the first week and the fourth week of life with a considerable increase in cells following this period. For example Shapiro and Bissen¹⁰ found that on the first day of life the percentage of myeloid cells (neutrophils, myelocytes and myeloblasts) average 61 per cent and nucleated red cells 32 per cent. On the eighth day the myeloid percentage increases to 77 per cent whereas the erythroid elements drop sharply to 12 per cent. At about 2 months of age as the marrow erythroid activity returns the peripheral blood shows an increase in reticulocytes and a rise in hemoglobin.

Blood Volume The blood volume of the full term infant at birth is approximately 85 ml per kilogram and that of the premature infant averages 108 ml, the difference being due mainly to an excess of plasma in the latter.¹¹ Normal blood volumes of 75 to 80 ml per kilogram which are the values in the adult are reached after the second month. In the treatment of erythroblastosis the blood volume for both full term and premature infants is calculated on the basis of 85 ml of blood per kilogram.

Hemoglobin Concentration The cord blood hemoglobin varies approximately between 16.6¹² and 17.1 gm per 100 ml of blood.⁷ In our laboratory the hemoglobin averaged 16.4 gm per 100 ml with a range from 14 to 19 gm per 100 ml.

Gardner and associates compared the composition of cord blood at birth with venous blood and concluded that the elevation of the hemoglobin level during the first day of life is due to the shift of fluid (namely whole plasma) from the circulation and is independent of placental blood transfer. The postnatal rise in hemoglobin and packed cell volume effected by the shift of fluid from the vascular compartment takes place within a few minutes after birth and is complete within an hour or two reaching a value of 19.1 gm per 100 ml of hemoglobin (S.D. 2.36) in one to eight hours (as compared with 16.6 gm in cord blood). The extent to which placental blood transfer contributes to this rise has been controversial.

According to Haselhorst and Altmann the average amount of blood in the placental vessels is 105 ml with a range from 50 to 200 ml. Infants receive varying amounts of this blood depending upon the time after delivery at which the cord is clamped. 51 per cent of the blood reaches the infant in the first minutes after delivery, 79 per cent in five minutes and 91 per cent in the first ten minutes. It is usually estimated that the placenta contains a reservoir of 125 to 150 ml of blood and that delayed clamping may account for the addition of as much as 100 ml of blood to the circulation of the newborn infant. It is claimed that the increased red cell and hemoglobin mass resulting from late clamping becomes apparent after birth only as the plasma volume is readjusted and equalized. DeMush and associates observed

that hematocrit values were higher for the infants whose placental blood had been allowed to pass into their circulation at birth averaging 61 per cent on the first day and 60 per cent on the third day. In those infants who failed to receive placental blood the hematocrit values averaged 50 per cent on the first day and 51 per cent on the third day practically the same as the average hematocrit in which of the umbilical cord blood which was 51 per cent. It seems most likely that the placental transfusion of substantial amount of blood by late clamping of the cord should be reflected in an elevation in hemoglobin soon after birth. In another study²² no significant difference was found in erythrocyte values in the neonatal period with respect to the time of clamping unless the cord was deliberately stripped.

Blood samples from heel puncture show a higher concentration of hemoglobin than those from the vein. This may be largely due to the stasis in the peripheral blood of newborn macrocytic red cells. Mollison and Cutbush¹⁶ give the following comparative normal hemoglobin values in newborn infants with cord blood hemoglobin of 13.6 to 19.6 gm per 100 ml the venous blood on the first day increases to 14.5 to 22.5 gm with corresponding skin prick values of 15.4 to 22.8 gm. These variations are important in the management of erythroblastosis (see Chapter 10).

The drop in hemoglobin to 11 gm per 100 ml of blood in the first two years of life is followed by a gradual rise reaching a maximum at 14 years of 16 gm for males and 14 gm for females with an average of 15 gm for both sexes.

Erythrocyte Count The red cell count is high at birth and averages 5.5 million with a range from 5 to 6 million per cubic millimeter. The red cells are macrocytic at birth. They range in size from 8 to 9 microns in diameter decrease to their smallest size of about 5 microns after three to six months and rise to adult dimensions of 7.2 to 7.5 microns at eight months.

The red blood cells average 4.6 million at the end of the first year and 4.8 million at the end of twelve years. At 14 years of age and over males average 5.4 million red cells per cubic millimeter and females average 4.8 million.

Abnormally high erythrocyte values of unknown etiology were reported at birth in an infant with anorexia lethargy cyanosis and convulsion.²³ The clinical signs subsided when blood was withdrawn and replaced with plasma. No congenital abnormalities were found. The etiology of this syndrome is unknown. In another case polycythemia in one twin and anemia in the other occurred in single-ovum twins with hemoglobin values of 20.2 and 3.7 gm and red cell counts of 7.47 and 1.85 million per cubic millimeter respectively. This discrepancy was explained on the basis of an arterio-venous shunt between the supposedly separate placental circulations which resulted in a partial systemic circulatory system. Unequal functioning between the two circulations accounted for the sharp differences in blood levels. In this case withdrawal of blood from the polycythemic infant was followed by an uneventful course. Usually newborn polycythemic levels drop to normal spontaneously and without ill effect to the infant. In another newborn infant polycythemic levels with a maximum of 7.5 million red cells per cubic millimeter persisted for six weeks before a spontaneous drop to 5 million red cells occurred.²⁴ Erythremia or polycythemia vera is not known to occur in infancy.

Hematocrit Percentage (Volume of Packed Red Cells) The hematocrit obtained after centrifugation of a given amount of blood averages 55 per cent at birth declines to 30 per cent by the second month increases to 36 per cent at 1 year of age and 40 per cent at 3 years of age and achieves normal adult values of about 45 per cent for males and 42 per cent for females in adolescence. Normal

children occasionally reveal a marked elevation of hematocrit red cells and hemoglobin during adolescence. These changes simulating polycythemia are transient and benign, revert to normal in the postadolescent period and require no treatment.

Size of Red Cells (MCV) and Hemoglobin Concentration (MCHC) The red cells are unusually large at birth with an average in the cord blood of 113 cubic microns and a range of 90 to 124 cubic microns.⁸ They diminish in size to a value of 69 cubic microns at 1 year of age.⁹ The lower limit of normal of 80 cubic microns for children and adults is reached at 4 to 5 years.⁸ The adult MCHC of 34 per cent is reached at 4 years of age.

Reticulocytes According to Windle,⁷ there are about 90 per cent reticulocytes in the blood of the human fetus at 3 months gestation and 15 to 30 per cent at 6 months gestation. The increased number of reticulocytes at birth reflects active red cell formation existing in fetal life.

Reticulocytes number 4 to 6 per cent at birth and remain approximately constant for about three days after birth. From the first half of the fourth day to the first half of the sixth day of life there is a pronounced drop. From the sixth to the seventh day there is a further and slower decrease to approximately adult levels (0.5 to 1.5 per cent).¹⁰

Normoblasts Normoblasts are frequently observed in the normal infant on the first day of life but usually disappear during the first week in most instances from the third to the fifth day. The average number at birth ranges from 3 to 10 per 100 white blood cells. In the premature infant the number of nucleated cells in the cord blood range from 10 to 20 per 100 white blood cells with higher figures prevailing in the smaller infant. Occasional nucleated red cells will be observed in the peripheral blood of an older infant with acute infection associated with anemia. The presence of normoblasts in the blood of the older infant and child with leukopenia, granulocytopenia and moderate anemia is indicative of bone marrow embarrassment and frequently invasion of the marrow by leukemic cells.

Platelets Variable numbers of platelets have been given for the neonatal period. Some workers have found fewer platelets during the first forty-eight hours than in older infants. Figures reported at time of birth range from 150,000 to 350,000 per cubic millimeter with an average of 300,000 at 2 weeks of age. The smaller number of platelets at birth has been attributed to the trauma incident to delivery. The rise in platelets is supposedly slower in the premature infant. The platelets of the newborn baby also show greater variation in size and shape than those in adults in whom larger platelets are found in appreciable numbers. The adult values of 250,000 to 350,000 per cubic millimeter of blood are reached at about 6 months of age.

White Blood Cells The total leukocyte count is high at birth ranging from 9,000 to 38,000 per cubic millimeter during the first two days of life with an average of 22,000 cells at the end of twelve hours.¹ In the first week the white blood count drops to an average of 12,000 per cubic millimeter with a range of 5,000 to 21,000. At the end of the first year the white blood cell count averages 12,000 with a gradual decline to values of 8,000 to 10,000 by the fourth year. The

total white blood cell count at birth tends to be lower in the premature infant than in the full term infant

Neutrophilic Leukocytes Neutrophilic leukocytes average 60 per cent at birth with a range from 40 to 50 per cent and include a small percentage of myelocytes By the tenth day the average drops to 40 per cent and then to 30 per cent in the fourth to the sixth month It remains at this level until the fourth year when a rise to 40 per cent takes place In the sixth year there is a rise to 55 to 60 per cent

Lymphocytes Lymphocytes average 30 per cent at birth and rise to 60 per cent in the fourth to sixth month These values are maintained until the fourth year when they drop to 50 per cent They drop to 40 per cent at the end of the sixth year and to 30 per cent by the eighth year

Monocytes Monocytes number 6 per cent at birth and except for a rise to 9 per cent in the second and third weeks remain at levels of about 5 per cent during infancy and childhood

Eosinophils and Basophils Eosinophils and basophils maintain levels of 2 to 3 per cent and 0.5 per cent respectively throughout infancy and childhood

Plasma Cells These are large spherical cells with deep blue cytoplasm containing vacuoles of varying size The nucleus eccentrically placed is round or oval with dense chromatin masses arranged like spokes in a wheel Plasma cells are not found in the embryo but appear after birth in interstitial lymphoid and glandular tissue Clinical evidence suggests their relationship to immunity because of their presence in patients with subacute and chronic infections Increased numbers of plasma cells have been noted in patients with infections in whom hyperglobulinemia is found On the other hand patients with α -immunoglobulinemia regularly exhibit a deficiency of plasma cells in hematopoietic centers and in inflammatory exudates¹

Summary of White Blood Cell Changes Certain approximate values may be designated for comparative purposes At the end of the second month of life leukocytes total 12 000 per cubic millimeter with 35 per cent granulocytes and 60 per cent lymphocytes These values are maintained until the fourth and fifth years when the total count drops to 9 000 white cells per cubic millimeter and the differential percentage is reversed to 60 per cent granulocytes and 35 per cent lymphocytes From the sixth to the fourteenth year there is a gradual numerical shift to adult values of 7 000 white blood cells per cubic millimeter with 65 per cent neutrophilic granulocytes and 30 per cent lymphocytes New born infants with total leukocyte counts at either extreme should be investigated for existing abnormalities There is evidence that premature infants as compared with full term infants show higher granulocyte lower lymphocyte and lower white blood cell counts

Blood of Premature Infants At the end of the decline in the second and third months the premature infant shows hemoglobin levels of 1 gm or more (depending upon the birth weight) below the values of 11 gm for full term infants and red blood cell counts below the average of 3 800 000 for full term infants Thereafter a gradual recovery sets in with values approximating normal full term levels at about 1 year of age The number of platelets may be somewhat lower at birth

in the premature infant than in full term infants averaging about 190 000 per cubic millimeter at birth and rising to 300 000 at the sixth month. The total number of white cells may be lower and nucleated red cells may persist for longer periods than in the full term infant. *Polycythemia* at birth may be exaggerated in the premature infant as compared with the full term infant because of differences in the proportion of the volume of blood in the body to that in the placenta. Whereas the amount of blood in the placenta remains fairly constant during the third trimester of pregnancy that in the body increases steadily. The effect of the transfer of placental blood in the infant after birth therefore would produce a greater relative effect in the premature infant than in the full term infant especially when tying of the cord is delayed and the full complement of blood is obtained from this source.

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Blood Dyscrasias in Relation to Maternal-Fetal Interaction

The pathogenesis and interpretation of many of the blood dyscrasias characteristic of infancy and childhood depend upon a consideration of maternal fetal relationships environmental and genetic influences and normal developmental changes. The problems of diagnosis and therapy often hinge on an understanding of many of the interrelated mechanisms that play a role in the control of the formation and development of blood cells.

Hereditary Basis of Blood Diseases—Genetic and Environmental Influences

Many of the blood disorders in the pediatric age group are conditioned by the overlapping influence of heredity and environment. Congenital defects may be genetically determined at the moment of conception or acquired during fetal development due to stress in the mother during critical moments of gestation. Warkany^{4, 48} postulated that the repeated appearance of defects in a family indicates the influence of a dominant or recessive inheritance of abnormal genes or of the repeated exposure of the developing embryo to the same adverse environmental factors. The majority of developmental defects are caused by a combination of circumstances involving both genetic and environmental factors.^{11, 12}

Blood Dyscrasias in Relation to Congenital Anomalies—General Principles

A growing body of evidence has been accumulating¹ in support of the association of a variety of disease states in the mother and the occurrence of abnormalities in the offspring. In the elaboration of these concepts more intensive investigation has also been directed toward genetic and familial aspects of a variety of diseases in which many of the blood disorders have been included.

The experimental production of developmental defects has prompted consideration of the origin of anomalies in every system. According to current interpretation single or coexisting anomalies represent deviations from orderly development induced by a known infection such as rubella or other as yet unidentified agents or states acting upon the pregnant woman and the placenta. If one accepts the principle that for all organs injury is most likely to occur during the stage of active differentiation the vulnerable period of erythropoiesis would extend from the sixth to the twelfth week when the liver spleen and

bone marrow are simultaneously involved in the proliferation of erythroblasts. Chronic thrombocytopenia present from birth may be related to suppression of platelet formation from the second month of fetal life when platelet production becomes evident.¹¹

It is thus apparent that the type of deformity depends upon nonspecific noxious influences operating at "developmental moments" corresponding to periods when organs are in their most rapidly proliferating condition. The infrequent association of severe types of hematologic disorders¹² with other congenital malformations suggests the relative resistance of hematopoietic tissues in comparison with the vulnerability of other systems during critical periods of development. However, the demonstration of injury to single or multiple cell types in the marrow in combination with other well-defined somatic defects infrequent as it appears supports the hypothesis of embryonic hematopoietic disturbances as a causative factor in blood dyscrasias.

Examples of blood dyscrasias associated with congenital anomalies include congenital leukemia and mongolism; hypoplastic aplastic anemias with skeletal defects (that is, Fanconi syndrome); sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations; and congenital labile factor deficiency with syndactylism.

Hematologic Aspects of Maternal and Fetal Interaction—Placental Physiology and Defects. The bulk of the placenta consists of chorionic villi which are exposed to a collection of maternal blood. Fetal and maternal blood are separated from each other by only two layers of cells at the trophoblastic endothelial junction of each villus. The maternal blood constantly circulates between the individual villi from which it is drained by the decidual sinuses. Microscopic defects or breaks in the villi conceivably could permit leaks of small amounts of fetal blood into the maternal circulation causing mild to moderate anemia in the newborn infant¹ as may be observed in Fig. 1.

Maternal arterial blood enters the intervillous space at a pressure of 60 to 70 mm Hg higher than the already existing pressure in this area and is dissipated by lateral dispersion. On the other hand, the difference between the umbilical arterial blood pressure and that in the intervillous space creates a pressure gradient conducive to the passage of water and metabolites from fetus to mother. It is conceivable that fetal blood may follow a similar course especially through breaks or infarcts in the villi resulting in neonatal anemia exclusive of incompatibilities.¹⁰ These observations suggest a mechanism by which an Rh negative woman is sensitized by an Rh positive fetus (namely, by the transplacental passage of fetal red blood cells or their products).

Fetal Hemorrhage Into the Maternal Circulation (Nonhemolytic Anemia of the Newborn Infant). Wiener⁴ was the first to suggest that the fetus can become severely anemic from bleeding into the maternal circulation. This concept was confirmed by Chown¹³ who reported a case in which a high concentration of fetal hemoglobin as well as fetal red cells could be found in the maternal blood soon after delivery. This suggested to him that anemia in the infant resulted from bleeding into the maternal circulation. The subsequent disappearance of fetal hemoglobin from the maternal circulation coincided with the disappearance

of the infants D (Rh₀) positive cells. It was calculated that approximately 90 to 180 ml of fetal blood had entered the maternal circulation sometime before birth.

When transplacental bleeding from the fetus occurs appropriate agglutination and serologic tests reveal that the mother has been the recipient of erythrocytes which differ in the ABO and Rh blood groups from her own erythrocytes and which are identical with those of the newborn infant. Also an elevated concentration of bilirubin and fetal hemoglobin in the mother's circulation immediately postpartum and their subsequent disappearance constitute further proof of the

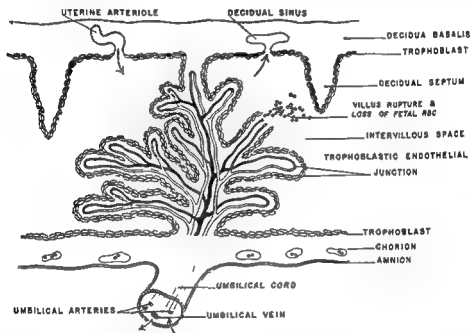


Fig 1 Schematic diagram of the placental circulation showing rupture of a villus at the trophoblastic endothelial junction permitting the mixing of fetal and maternal blood and the entrance of fetal red blood cells into the maternal circulation (Modified from Javert C T and Reiss C. Origin and Significance of Macroscopic Intervillous Coagulation Hematomas [Red Infarcts] of Human Placenta. *Surg Gynec & Obst* 94:257 1952)

passage of fetal blood into the maternal circulation through breaks of varying size in the placental barrier. The finding of increased fetal hemoglobin levels in the mother's blood which gradually diminish provides evidence for the leakage of fetal blood when the ABO and Rh blood groups in the fetus and the mother are identical.³

Additional evidence for the entrance of fetal antigens into the maternal circulation just before delivery is the reported appearance in group O mothers of immune anti A antibodies (from A infants) ten to twenty days after delivery when none had been present at birth.¹⁰

The loss of fetal blood into the maternal circulation has been described in

many reports on the basis of an excess of alkali resistant hemoglobin and differential agglutination of red cells in maternal blood^{21 26 27 28} Iron deficiency anemia has been reported as one of the complications of occult blood loss due to fetal maternal transfusion.⁴

Symptomatology and Blood Findings—An evaluation of these reports indicates that the leakage of fetal blood into the maternal circulation can occur in small amounts over a prolonged period or may take place in larger amounts shortly before delivery or during labor. Severe anemia and shock in the immediate postnatal period indicate transplacental hemorrhage from fetus to mother just prior to delivery.²¹ Pallor is a significant feature jaundice is absent. The spleen and liver are not enlarged. Anemia of moderate or severe degree with a hypochromic microcytic blood smear characteristic of iron deficiency may be present. If severe hemorrhage occurs at the time of delivery, the cord blood hemoglobin may be normal due to hemoconcentration. The evidences of anemia become perceptible with later hemodilution. Leukocytosis, reticulocytosis and nucleated red cells are noted occasionally, but hyperbilirubinemia is unusual in contrast to erythroblastosis.¹

The mother may experience no symptoms during pregnancy from fetal bleeding into her circulation. When the mother is the recipient of incompatible blood from the fetus, the symptomatology is analogous to a transfusion reaction with chills, fever and hyperbilirubinemia.¹

Treatment—Transfusions with packed cells sufficient to bring the hemoglobin to approximately 12 gm per 100 ml are advisable in severe anemia. Since the loss of blood implies a decrease of potential iron stores, supplemental oral iron medication is advisable during recovery.

Bleeding From the Placental Surface—A significant number of newborn infants are born anemic not only because of occult hemorrhage from the passage of fetal blood into the maternal circulation but also because of serious fetal blood loss during labor and delivery. In abruptio placentae and placenta previa, bleeding takes place from the placental surface in the area of its attachment to the uterine wall. Blood loss suffered by the mother is shared by the infant with resulting posthemorrhagic anemia and shock (asphyxia pallida) and even stillbirth. Similar consequences follow intrauterine rupture or tear of fetal vessels during labor.²⁷ Exsanguination from rapid blood loss occurs in precipitous deliveries from velamentous insertion of the cord and less commonly from rupture of a normal or abnormally short cord.^{19 20} Treatment is urgent and consists of clearing the airway and administering oxygen, artificial respiration and transfusions with packed red cells.

Placental Transmission of Antibodies and Isoagglutinins—Since from an anatomic point of view, the maternal and fetal circulations in man are separated by only two layers of cells, placental transmission of a variety of substances including maternal antibodies, isoagglutinins, plasma proteins and steroids is effected in the human infant. Antibodies and gamma globulin are not produced in significant amounts in the fetus of any species under normal conditions. Their presence in the fetus and newborn infant prenatally results from transfer across the placenta from the mother. Postnatally these substances are derived from the

of the infants D (Rh₀) positive cells. It was calculated that approximately 90 to 180 ml of fetal blood had entered the maternal circulation sometime before birth.

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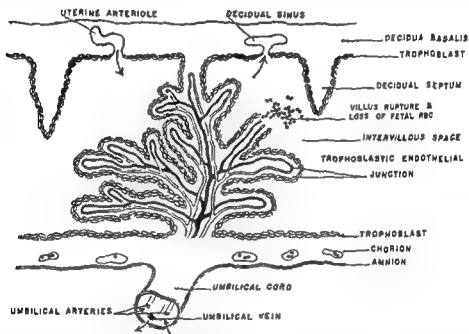


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Additional evidence for the entrance of fetal antigens into the maternal circulation just before delivery is the reported appearance in group O mothers of immune anti A antibodies (from A infants) ten to twenty days after delivery when none had been present at birth.¹⁰

The loss of fetal blood into the maternal circulation has been described in

with the number of cases in which the factor presumably appears in the newborn infant by passive transfer

Plasma Proteins in the Fetus and Newborn Infant The gamma globulin in the cord blood and in the blood of the newborn baby (600 to 1,200 mg per 100 ml) is derived entirely from the maternal circulation by placental passage. Low gamma globulin levels are found in fetal blood until the fourth or fifth month of pregnancy after which they rise gradually to reach maternal concentrations by the eighth to ninth month. At birth the gamma globulin level in fetal blood is somewhat higher than in maternal blood.⁴¹ The gamma globulin is observed to fall steadily in the first month of life following a simple exponential curve reaching one third of the birth value at 1 month of age when it is stabilized.^{3,4} No change occurs between 1 and 3 months of age (300 to 600 mg per 100 ml) when the rate of synthesis balances the rate of catabolism. From 3 months of age levels of gamma globulin rise slowly and progressively and reach adult levels (900 mg per 100 ml) at 2 years of age.¹ According to these calculations the half life of gamma globulin is approximately twenty five days^{31,34} which is comparable to the thirty-day half life of the Rh antibody molecule which passes through the placenta.⁴¹

Dancis and associates have shown that under normal circumstances the placenta does not contribute significantly to the plasma proteins of the fetus. Beginning early in gestation the fetal liver is capable of synthesizing all plasma proteins with the exception of gamma globulin. Little gamma globulin can be detected in fetal plasma up to six months of gestation.³⁹ It is problematic whether the infant normally synthesizes gamma globulin before the fourth week of extrauterine life. Probably no significant amounts are synthesized before the age of 3 months.

Mean values for total serum protein are lower in the cord blood of newborn infants than in their mothers at term. The lowering is a reflection of the lower globulin fraction since the mean value for serum albumin is much higher in the newborn infant than in the mother at term. After birth the level of serum globulin falls steadily reaching its lowest level at 3 to 4 months of age and then rising to adult levels by 7 to 11 months of age.³¹

Immunologic Relationships The following immunologic relationships exist

Erythroblastosis Fetalis The maternal fetal relationships in relation to sensitization by antigens of the Rh positive fetus and in cases of a heterospecific pregnancy of the A, B and O groups will be dealt with in another chapter (Chapter 9). They represent important examples of antibody relationships and immunosensitization between maternal and fetal blood.

Thrombocytopenic Purpura in the Newborn Infant Hemorrhagic disease of the newborn infant and congenital thrombocytopenic purpura make their appearance soon after birth and reflect disturbances resulting from maternal fetal relationships. Thrombocytopenic purpura in the newborn infant may be cited as another example of the influence of maternal suppressing factors on a fetal blood element. This disease affects children whose mothers either have thrombocytopenic purpura or are entirely normal. In the former instance the reduction of platelets in the infant results from the passage across the placental membrane

ingestion of colostrum and maternal milk.⁴¹ This contrasts with the more difficult transmission in the ruminant in which there is a complex placental structure (five tissue layers between maternal and fetal circulation at full term of gestation). Therefore, as knowledge of placental function has been extended it has become increasingly clear that a wide variety of substances including proteins and red cells cross the placental barrier into the fetal circulation and also that the rate of transfer is selectively controlled.⁶

While A and B isoenzymes can be demonstrated in the fetus in the second and third months,⁴² isoenzymes appear later. In a majority of newborn infants isoenzymes detected in the cord blood diminish in titer or completely disappear. Consideration of the similarity existing between the blood of the baby and that of the mother with respect to isoenzymes suggests the passive transfer of isoenzymes from the maternal into the fetal circulation through the placenta.⁴³ This process is analogous for example to the transplacental passage of anti Rh antibodies, gamma globulin, diphtheria and tetanus antitoxin and immune bodies for measles and mumps virus from mother to child.

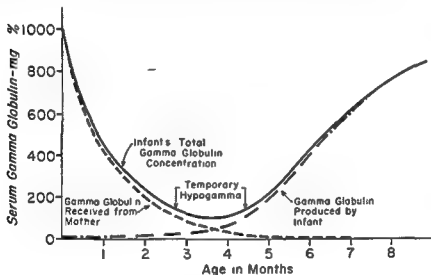


Fig 2 Graph showing levels of gamma globulin in physiologic hypogammaglobulinemia of newborn infants. As level of gamma globulin from mother drops and production by infant begins there is a temporary drop in gamma globulin concentration. (From Burrett B and Volwiler W: Agammaglobulinemia and Hypoglobulinemia. JAMA 164:866, 1957.)

Placental Transmission of the LE Factor Cases of systemic lupus erythematosus in pregnancy have been reported in which the LE phenomenon has been demonstrated in the newborn infant.⁴⁴ The LE factor was present in the cord blood and persisted for approximately seven weeks after delivery, a period corresponding with the half life of gamma globulin with which it is identified. (See discussion on LE phenomenon Chapter 18.) The babies remained healthy; this was considered an indication that the LE factor is derived by transplacental passage from the mother rather than by an immunologic process developing in utero. The inheritance of systemic lupus erythematosus is rare⁴⁵ in comparison

since it cannot be replenished once the maternal source is eliminated except in circumstances in which it can be conveyed by colostrum or maternal milk

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of a circulatory factor which depresses platelet formation in the infant and causes purpura. Platelet autoagglutinins have been found in the blood of the mother and infant. Isoagglutinins for each others platelets have been found also.³ These agglutinins disappear from the infants circulation by the age of 3 months.

Fetal Maternal Leukocyte Incompatibility That leukoagglutinins may develop in multitransfused patients has been recognized. Leukoagglutinins are believed to be comparable to antibodies for erythrocytes and platelets. Antigenic differences in human leukocytes are known and leukoagglutinins have been held responsible for febrile transfusion reactions.³³ Fetal maternal incompatibility may induce leukoagglutinin formation in the mother who has had no transfusions when the antigenic factor has been inherited from the father and is absent in the mother.³⁴

Transient agranulocytosis has been observed in successive siblings in the neonatal period.⁴ Such a circumstance was explained by transplacental isoimmunization of the mother to a leukocyte factor of her infant in a manner analogous to Rh isoimmunization causing hemolytic disease of the newborn infant. In these cases agranulocytosis persisted for three to four weeks and in one of the infants it was accompanied by pulmonary infection. The predominance of neutrophilic myelocytes in the bone marrow in these infants may represent either a maturation arrest or a depletion of mature cells because of their increased agglutination and destruction in the peripheral blood.

Two infants born of mothers with chronic neutropenia showed a transitory neutropenia which persisted for three weeks.⁴⁰ The blood picture in the babies was presumably caused by the transplacental passage of a neutropenic factor. In one case the mothers serum contained a demonstrable leukoagglutinin which could be transferred by transfusion to a normal person. In these patients the bone marrow showed hyperplasia of the granulocytic series with a maturation arrest at the myelocytic stage.

Transplacental Passage of Drugs Affecting Blood Elements in the Newborn Infant Several examples may be cited of the passage of drugs through the placenta affecting the blood elements in the newborn infant. In one case report the administration of quinine to induce labor resulted in thrombocytopenia in the mother and infant. The mechanism was based on the transmission of quinine platelet antibodies derived from the mother who had originally received the drug in childhood. The preterm administration of a large dose of a vitamin K analogue (Hykinone) to mothers during labor has also been shown to result in marked hyperbilirubinemia in newborn premature infants with nervous system involvement in several cases.³ The increased pigment presumably resulted from the hemolytic effects of the vitamin. In another case¹⁶ the ingestion of moth balls by a mother produced jaundice and hemolytic anemia in her newborn infant. This was attributed to the passage of naphthalene and its derivatives to the infant.

Generally substances capable of injuring the fetus and newborn infant which have been transmitted by transplacental passage from the mother play a passing role in producing pathologic states. The noxious agent has a limited activity

Erythrocytes—General Considerations

Properties of the Erythrocyte The essential morphologic and physiologic aspects of the erythrocyte are as follows:

Erythron The term erythron refers to the tissue made up of the circulating red blood cells and their precursors in the bone marrow. It conveys a sense of functional unity to a series of cells ranging from the early erythroblast to the non-nucleated red corpuscles.

Erythrocytes—Structure and Function Normal human red blood cells are non-nucleated biconcave discs whose primary function is the transport of hemoglobin, which represents 34 per cent of their fresh weight. They are structurally adapted for rapid movement throughout the circulation to bring oxygen from the lungs and distribute it rapidly to the tissues through the smallest capillaries. The red cell is thicker at the periphery than in the central portion and possesses an elasticity which facilitates mechanical buffeting and movement through capillaries narrower than its own diameter. Collectively, the red cells offer an extensive surface of approximately 3,000 square meters in the adult to facilitate the absorption and discharge of oxygen and perhaps contribute to the movement of other substances by surface absorption. Hemoglobin is not held in solution but adheres closely within the meshes of stroma, a spongelike material filling the interior of the cell. The outer surface or envelope, which serves as a semipermeable selective membrane in the passage of substances in and out of the cell, is composed principally of lipids consisting of a protein, stromatin, in combination with lecithin and cholesterol. The red cell surface possesses blood group properties, affinities, and specificities involved in hemolysis and agglutination.

In addition to hemoglobin, which constitutes more than 90 per cent of solids in the red cell, contains water, protein, lipids, carbohydrates, vitamins, iron, and a group of enzyme systems which maintain the integrity of the cell membrane. In the circulation, hemoglobin remains relatively static, whereas other red cell constituents undergo constant physical and chemical changes relating to their metabolism.¹⁹

It has been pointed out that in addition to the structural proteins there are approximately forty enzymes thus far identified in the human red cell.²⁰ Among these are the specific enzymes

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Porphyryns and Blood Disorders The porphyrins are red pigments with a pyrrole structure which serve as precursors of protoporphyrins I and III and of their corresponding coproporphyrins. Both coproporphyrins are excreted in excess in urine and stool in certain blood disorders: type I in pernicious anemia, congenital spherocytic anemia, and leukemia, and type III in aplastic anemia and Hodgkins disease, and after administration of heavy metals such as mercurials and arsenicals. The examination of the urine for porphyrins is especially important in lead poisoning. The greatest increases in coproporphyrin excretion occur in this condition although this may not be a consistent finding. Splenectomy may be followed by diminished porphyrin formation in the bone marrow. Free protoporphyrin normally found in small amounts in erythrocytes is greatly increased in the blood of patients with iron deficiency anemia and disorders in which utilization of iron in hemoglobin synthesis is interfered with such as in anemia of infection.

Rouleaux Formation and Sedimentation In a fresh preparation on a slide red cells tend to cluster together with their broad surfaces in contact forming a grouping called rouleaux. The disproportionate thickness and difficulty of approximation of the red cells in congenital spherocytic anemia results in loss of rouleaux formation and represents a diagnostic feature of this disease.

In conditions associated with rapid sedimentation the initial phase consists of increased rouleaux formation; the larger the aggregates the more rapid is the sedimentation rate. Accelerated rates are primarily influenced by increases in fibrinogen and to a large extent by globulin in the plasma.

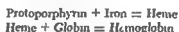
Electrolyte Considerations The electrolyte composition of red blood cells is qualitatively similar to the plasma except for the predominance of potassium in the former and sodium in the latter. In the passage of inorganic ions in and out of the cell the red cell membrane acts as a dynamic rather than as a passive semipermeable structure. The energy for this and other functions of the mammalian cell is derived from the breakdown of glucose into lactic acid and from the presence of nucleosides such as adenosine triphosphate (ATP). In normal metabolic activity of the red cell membrane a high concentration of potassium is maintained within the cell and sodium is excluded. In blood stored under refrigeration glycolytic activity is inhibited and potassium diffuses out of the cells into the plasma and sodium enters.⁴⁶ The accumulation of potassium in the donor plasma is an important consideration in exchange transfusions in erythroblastosis and can conceivably result in increased serum potassium levels in the infant. With normal kidney function however this state rarely develops. On the other hand the too rapid infusion of hyperkalemic donor blood that is not fully warmed carries the potentiality of untoward toxic effects. Since the metabolic activity is restored by exposure to 37° C and results in return of the intracellular potassium, the use of fresh warm blood is recommended.^{4,34} It has also been found that the ability of stored red cells to regain potassium when they are warmed depends upon the presence of adenosine and that the addition of this substance and glucose is needed for optimal red cell preservation.^{11,12}

Adsorption by the Surface of Red Blood Cells In the relation between the red cells and their environment the occurrence of adsorptive phenomena is to be

linked with the multiple activities of the red cell in supplying energy from glycolysis. Another example of the mediation of enzymes is the increased susceptibility of the cell to hemolysis caused by primaquine sensitivity due to a low glutathione level of the red cell and a deficiency of glucose 6 phosphate dehydrogenase activity.

The normal red blood cell has a volume of 65 cubic microns and contains 25 to 30 micromicrograms of hemoglobin. The red cells macrocytic at birth diminish in size during the neonatal period and reach normal sizes of 72 to 75 microns in diameter by 8 months of age.

Hemoglobin—Structure and Function Hemoglobin the coloring matter of erythrocytes is a conjugate of a pigment (heme) and a protein (globin). The heme component consists of a porphyrin (the union of four pyrrole groups) in combination with iron in the ferrous state. The hemes are pigments which account for the characteristic red color of hemoglobin. The particular porphyrin of hemoglobin is structurally a protoporphyrin. In the synthesis of heme protoporphyrin is elaborated from the interaction of glycine and "active" succinate.⁹ In vitro evidence suggests that the biosynthesis of heme from iron and protoporphyrin is enzyme dependent.⁴ Hemoglobin may then be regarded as a ferrous protoporphyrin with the following composition:



Heme is also found in myoglobin (muscle hemoglobin) and in the respiratory enzymes such as the cytochromes. The basic function of the heme compounds is to transport oxygen and make it available to the cell. Hemoglobin combines loosely with oxygen to form oxyhemoglobin; this unstable association permits the diffusion of oxygen to tissues for oxidative purposes. The iron of hemoglobin remains in the ferrous state throughout respiration. Methemoglobin, on the other hand, is the oxidized heme formed from reduced hemoglobin in which the iron is in firm combination in the ferric state and is incapable of functioning as an oxygen carrier. Globin, the protein component of the hemoglobin, is synthesized from the amino acids of destroyed protein and from the globin of effete red blood cells. For the complete synthesis of globin an adequate intake of protein is required.

Iron Content and Oxygen Capacity Hemoglobin contains 0.335 per cent of iron so that 1 gm. may be said to contain 3.4 mg. of iron. Also 1 gm. of hemoglobin unites with 1.34 ml. of oxygen. Oxygen capacity depends upon hemoglobin concentration. Since the normal hemoglobin in the older child and adult is approximately 15 gm., the oxygen capacity is therefore 20 ml. per 100 ml. of blood or 20 volumes per cent. The degree of oxygen saturation of blood is derived from the ratio of oxygen content (19 volumes per cent in arterial blood) to oxygen capacity and is normally 95 per cent.

Cyanosis depends upon the absolute amount of reduced hemoglobin in capillary blood and not upon the content of carbon dioxide. It has been determined that capillary blood must contain at least 5 gm. of hemoglobin per 100 ml. of blood to reveal cyanosis. In severe anemia with values below this level the amount of reduced hemoglobin will therefore be insufficient to show cyanosis.

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Adsorption by the Surface of Red Blood Cells In the relation between the red cells and their environment the occurrence of adsorptive phenomena is to be

expected. The extensive surface presented by the red blood cells and its intimate contact with a diversity of substances in the plasma provide opportunities for adsorptive processes¹³ comparable to those which occur at other interfaces. This function of red blood cells is yet but little investigated may account in part for the transport of metabolic, immune and endocrine products through the blood stream. The quantity attached to the red blood cell however is probably minimal as compared with that free in the plasma and the substances fixed by the stroma are probably more slowly released than those in the plasma.

Osmotic Fragility Changes in osmotic equilibrium surrounding the red cells influence their size. At a concentration of 0.85 per cent a solution of sodium chloride is isotonic for the red cell so neither swelling nor shrinkage results. In a medium of hypotonic sodium chloride solution on the contrary fluid passes into the cells increasing their size and the cells lose their biconcavity and become spherical. As a critical level is reached more and more of the cells swell, burst and release hemoglobin. The first trace of hemolysis normally appears when sodium chloride concentration reaches 0.42 per cent and hemolysis is complete at 0.32 to 0.35 per cent.

The resistance of the red corpuscles to varying dilutions of hypotonic solution of sodium chloride constitutes an important laboratory procedure in the diagnosis of the anemias and is especially useful in the differentiation of the hemolytic group. The response of blood cells in the fragility test does not necessarily reflect their reaction within the circulating blood where conditions of isotonicity prevail but undoubtedly depends upon a number of intrinsic factors which involve the structure and properties of the cell envelope. However the test constitutes a useful gross index of the relative thickness of the major number of red cells in the sample of blood to be tested. If the red cell envelope is small in relation to its volume as in the thick red cells or spherocytes of hereditary spherocytosis the resistance is decreased. Increased resistance to hemolysis is noted when the bulk of red blood cells possess relatively larger surfaces in comparison with their substance as occurs in the thin red cells of patients with Mediterranean anemia and in patients with iron deficiency anemia. Sickle cells are also more resistant to hypotonic saline solution than are normal cells.

Studies of the fragility of red blood cells of the newborn infant led to equivocal results varying from decreased¹ to increased resistance. In our laboratory moderately increased resistance has been observed in the cord blood of the full term infant but is more marked in the premature infant. On the other hand a slightly increased or normal fragility has been found¹ in the cord blood of the normal newborn infant. In these studies it was noted that the erythrocytes of most children with congenital cyanotic heart disease had only slightly greater fragility than those of normal patients. These purely *in vitro* studies bear no relation to the shortened life span of fetal red blood cells when they are tagged with Cr^{51} and injected into normal individuals.

Mechanical Fragility Tests on mechanical fragility which evaluate the strength of the red cell membrane by exposing erythrocytes to the trauma of rolling glass beads under prescribed conditions are regarded as more closely simulating physiologic conditions prevailing in the circulation. With this test it was found¹ that

erythrocytes of the newborn infant break down more rapidly in the first days of life. This increased mechanical fragility which falls to normal by the fifth and sixth days was associated with a rise in serum bilirubin. Although red cells and hemoglobin levels show minimal quantitative fluctuations in the first days of life it is possible that shifts in plasma volume may obscure the rapid elimination of macrocytic cells existing during this period. According to this concept physiologic jaundice stems from at least two sources: a hemolytic component and hepatic immaturity. However, current emphasis has been placed on the latter factor as being of major importance.

Red Cell Thickness and Oxygen Dissociation. It has been demonstrated by animal experiments that thickness of the red cells bears a relation to oxygen dissociation. Thus the blood of patients with spherocytic cells is less able to deliver oxygen to the tissues than is blood of normal patients or of patients with anemias in which the red cells are reduced in thickness.¹⁷

Erythrocyte Production. Multiple factors many of which have specialized functions enter into the production of red cells.

Factors Required for Red Cell Production. Normal maturation of erythrocyte precursors requires amino acids, protein, and the B vitamins, especially pyridoxine, folic acid (pteroylglutamic acid), riboflavin, vitamin B₁₂, and the nucleic acid constituents thymine and thymidine. Of these factors, vitamin B₁₂, folic acid, and ascorbic acid are fundamentally involved in the development of the red blood cell. Folic acid participates in red cell maturation only after conversion, largely by ascorbic acid, to its biologically active form, folinic acid or citrovorum factor. Vitamin B₁₂ constitutes both the extrinsic factor and the erythrocyte maturation factor. The intrinsic factor from the stomach is necessary for the absorption of vitamin B₁₂. Where patients with pernicious anemia respond to either folic acid or vitamin B₁₂, those with megaloblastic anemia of infancy, in whom similar abnormal cells appear in the bone marrow, usually respond to folic acid alone but rarely to vitamin B₁₂. Both folinic acid (or folic acid) and vitamin B₁₂ serve as coenzymes in nucleic acid synthesis in the production of thymine and thymidine.

Factors required in red cell (stroma) formation and hemoglobin (heme and globin) synthesis overlap to the extent that the separate consideration of each process is not always feasible. Although the need for minerals applies more particularly to the elaboration of hemoglobin, many of the vitamins and amino acids are involved in both processes. For example, a deficiency in minerals results in both lowered hemoglobin levels and morphologic changes in the red blood cell. Many of the inherited abnormal hemoglobins are associated with the presence of such definitive changes in the erythrocytes as sickle cells and target cells. To overcome the need for a sharp division and to allow for this interrelationship, the term erythropoiesis is therefore frequently employed to cover both processes.

Humoral Regulation of Erythropoiesis (Hemopoietin or Erythropoietin). The physiologic balance between red cell production and destruction which results in a remarkable consistency of number and volume involves the participation of several regulatory mechanisms. These are manifested in the stimulation of erythro-

poiesis after hemorrhage and after exposure to an atmosphere of reduced oxygen tension

Bone marrow anoxia provides the primary stimulus for erythropoiesis as evidenced by polycythemia in chronic hypoxic states and the suppression of erythropoiesis when oxygen concentration is increased. It is not yet clear whether anoxia acts directly on the bone marrow or on the organism to produce a stimulating impulse. It is probable that lowered oxygen tensions stimulate bone marrow activity by a mediation of a humoral factor, hemopoietin or erythropoietin.

The ability of the serum and plasma of bled animals to induce a rise of reticulocytes, red blood cells, and hemoglobin when injected into normal animals gives further support to the presence of such a humoral factor. The existence of this factor was originally postulated in 1906 by Carnot and Delfandre, who transfused plasma from a bled animal into an intact animal. Reismann⁵ presented the first convincing evidence for the existence of a humoral factor by demonstrating similar hyperplasia of the bone marrow in a pair of parabiotic rats following chronic hypoxia induced in one partner. Erslev and Liviets^{1, 16} stimulated further interest in this mechanism by the bioassay of large amounts of plasma from bled rabbits and monkeys which had induced reticulocytosis and other evidences of increased erythropoietic activity of the bone marrow in normal animals.

In the human subject data have become available compatible with the humoral regulation of erythropoiesis. Plasma filtrates from patients with severe Cooley's anemia were found to stimulate hematopoiesis in intact rats, whereas similar preparations from normal subjects and one patient with chronic hypoplastic anemia were inactive.³⁷ Gurney and associates reported finding increased erythropoietin (erythrocyte stimulating factor activity) in the serum of two patients with congenital hypoplastic anemia.

The suppression of erythropoiesis after hypertransfusion^{5, 13} may indicate the mediation of humoral regulation in response to a lessened need for erythropoiesis. On the other hand, the similar depression of hematopoiesis following multiple transfusions⁴⁴ in chronically anemic patients suggests regulation in different ways or degrees from those involved in the normal individual. It appears possible that the physiologic adjustments in the chronically anemic person are such that hemoglobin concentrations which would be considered indicative of severe anemia under ordinary circumstances are in fact "normal" for that patient.

The effect of cobalt in increasing the rate of erythropoiesis in man and experimental animals to produce polycythemia has been attributed to bone marrow anoxia as a consequence of a toxic action on one or more enzyme systems. It has been shown that certain of the properties of erythropoietin in plasma of anemic patients are also common to those of the active factor in cobalt plasma, indicating that cobalt enhances red cell production by increasing the formation of erythropoietin.

The actual site where the erythrocyte stimulating factor is produced has not yet been clearly determined. Emphasis has been placed on the kidney as being, directly or indirectly, concerned with formation of the factor rather than the hemopoietic or other tissues.^{3, 7}

Erythropoietin is a protein found in highest concentrations in patients with diseases associated with erythroid aplasia.⁴ Significant amounts have been noted in congenital hypoplastic anemia, aplastic anemia, acute lymphoblastic leukemia, and chloramphenicol-induced bone marrow aplasia. In patients with hemolytic conditions associated with erythroid hyperplasia, as Cooley's anemia and sickle cell disease, significant levels of erythropoietin are found; however, they are found less frequently than in patients with the aplastic group of anemias. Low concentrations are observed in patients with uremia and chronic inflammation. The only sources of erythropoietin are the plasma and urine, but therapeutic use is limited until richer exogenous supplies become available. Although cobalt increases endogenous erythropoietin production, its use is handicapped by toxicity.⁴

Characteristics of Primitive Blood Cells: Primitive cells of both the red and white cell series possess similar structural characteristics and in their maturation reveal many points in common. The early blast forms are large, the cytoplasm is deeply basophilic, the nucleus occupies more space and stains less deeply than the cytoplasm (leptochromatic), the chromatin is finely granular and one or more nucleoli are present. At this stage classification is facilitated by comparison with more mature cells with which they are associated by noting morphologic similarities. Maturation is accompanied by the following features: the primitive cell in each series becomes progressively smaller in the content of cytoplasm and nucleus, the basophilia of the cytoplasm lessens, the chromatin becomes more condensed, its original purplish color changes to dark blue, and the nucleoli disappear early. In the red cell the basophilia is replaced by hemoglobin. In the mature granulocyte the cytoplasm becomes faintly pink with specific granulation; in the lymphocyte a bluish or sky blue color, and in the monocyte a gray blue with fine reddish blue granules.

Stages in Maturation of Red Blood Cells—General Principles: The normal progression of red cell maturation is based upon intracellular chemical changes. A knowledge of these changes aids in identification of individual cells.

NUCLEIC ACID AND CELLULAR DEVELOPMENT: Cellular growth and multiplication of cells are closely identified with the content of the nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Although they both occur together, the former predominates in the cytoplasm (especially in the mitochondria) and nucleolus and the latter in the chromatin of the cell nucleus. Deoxyribonucleic acid present in the nuclear material of all living cells is generally believed to be the principal component of genes. Rapid growth of all cellular types, especially during mitosis, is accompanied by increased concentrations of the nucleic acids.⁴

BASOPHILIA: Deep basophilia of the cytoplasm characterizes the stem cell and red and white cell precursors at the "blast" level of immaturity. The high content of ribose nucleic acid parallels the maximal cytoplasmic basophilia. The basophilic staining is ascribed to the affinity of nucleic acid in cytoplasm for the basic component (methylene blue) of Wright's stain or other polychromatic stains. In all cell types basophilia recedes with increasing maturity, and in the red cell its gradual replacement by hemoglobin results in an increased affinity for acid dyes (eosin).

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pernicious anemia and related megaloblastic anemia in five to seven days and following iron therapy in patients with iron-deficiency anemia in five to ten days.

Abnormal Maturation (Deficiency of the Hematopoietic Factors—Liver, Folic Acid, and Vitamin B₁₂) The cells are abnormally large and the nucleus matures more slowly than cytoplasm.

Promegakoblasts (erythrogonies)

Regarded by some as common progenitors of both pathologic megaloblasts and of normoblasts may be regarded as earlier form of megaloblast large cell. Cytoplasm abundant basophilic. Nucleus light purple staining chromatin open reticular pattern stippled no clumping as in pronormoblast several nucleoli present.

Megaloblast (basophilic megaloblast)

Corresponds to promegakoblast. Cytoplasm more intense dark blue basophilic. Nucleus no nucleoli chromatin finely divided.

Polychromatic Megaloblast (intermediate megaloblast)

Large cell. Cytoplasm hemoglobin now present and basophilia decreased resulting in multi-colored polychromatophilic cytoplasm. Nucleus purple still finely granular with occasional clumping.

Orthochromatic Megaloblast (late megaloblast)

Larger than corresponding late normoblast. Cytoplasm deeply eosinophilic. Nucleus purplish blue reduced in size irregular clumping but reticular appearance still present never cartwheel shaped.

Macrocyte

Larger than normal erythrocyte which it resembles measures 9 microns or more in diameter.

The megaloblast series in contrast to corresponding normoblasts shows less clumping and greater retention of the fine reticulated appearance of the nucleus during maturation and at each stage is larger than the corresponding normoblast. Cells with partial deficiency of the hematopoietic factor intermediate in nuclear structure between the megaloblast and normoblast appear in the bone marrow. With increased deficiency megaloblasts appear whereas specific treatment (such as folic acid in infantile megaloblastic anemia) results in differentiation into normoblasts.

Reisser²⁰ points out that the nuclear chromatin particles are more homogeneous in the normoblastic cell as compared with the diffuse particulate meshlike chromatin of the megaloblastic cells and that the ratio of mitotable (that is capable of mitosis) maturing cells is much increased in the latter. Megaloblasts are conceived as red cell precursors with a prolonged resting phase between mitoses allowing a longer time for the dispersion of the chromatin throughout the nucleus. The conditions most conducive to megaloblastic blood formation are states in which vitamin B₁₂ or folic acid is deficient. Normoblastic hematopoiesis results in normal erythrocytes as compared with the oval macrocytes of megaloblastic hematopoiesis. Megaloblasts are found in the bone marrow not only in pernicious anemia and megaloblastic anemia of infancy and of intestinal origin but also in a number of miscellaneous states in which for various reasons the patient has levels of vitamin B₁₂ and folic acid inadequate for the increased demands for hematopoiesis. These include multiple myeloma, hemolytic anemia, idiopathic aplastic anemia, chronic blood loss with superimposed acute hemorrhage, myelofibrosis, erythremic myelosis, advanced cirrhosis of the liver after anticonvulsant drugs (phenytoin sodium [Dilantin][®] and primidone [Mysoline][®] and leukemia in which antifolates and 6-mercaptopurine have been used).²¹

POLYCHROMATASIA AND STIPPLING Admixtures of basophilic substance and hemoglobin in intermediate stages produce *polychromatophilia* of the cytoplasm. Stippling (punctate basophilia) refers to the fine or coarse bluish violet granules found in the red cells. Stippled cells are noted in a variety of clinical conditions such as lead poisoning and hemolytic and deficiency anemias of varying severity.

ERYTHROPOIESIS Two types of red cell maturation can be described: the pathologic form associated with a deficiency of hematopoietic or liver principle, megaloblastic erythropoiesis, and the normal process, normoblastic erythropoiesis. The recognition of the various stages in either normal or abnormal maturation depends upon cell size, staining of the cytoplasm, and nuclear conformation according to the principles already stated. Only those cell types need be included here which are commonly observed in pediatric practice and can be identified by bone marrow aspiration. The difficulties in distinguishing between megaloblasts and early cells of the normoblastic series are confined to two conditions in this age period: megaloblastic anemia of infancy and the rare juvenile pernicious anemia. Other forms of anemia involve the orderly maturation of the normoblastic series. The term erythroblast has assumed a comprehensive connotation and includes all nucleated red cells, both normal and pathologic.

Normal Maturation It will be noted that in the normal maturation the cytoplasm and nucleus mature simultaneously and synchronously. The stages of normal red cell development and their chief morphologic and staining characteristics may be outlined as follows:

Pronormoblast (proerythroblast)

About twice the size of a normal mature erythrocyte. Cytoplasm narrow rimmed, deeply basophilic. Nucleus light purplish, vesicular, granular, slight clumping, one or more nucleoli present.

Basophilic normoblast (early normoblast or early erythroblast)

Smaller than pronormoblast. Cytoplasm basophilic. Nucleus darker purplish staining, chromatin clumping more marked, may be cart wheel, nucleoli absent.

Polychromatic normoblast (intermediate normoblast, late erythroblast)

Cytoplasm less basophilic, traces of hemoglobin present. Nucleus shrunk, more mature, chromatin bluish black, coarse clumped, a light area resembling hemoglobin often seen at one pole of the nucleus.

Orthochromatic normoblast (late normoblast)

Size somewhat larger than erythroblast. Cytoplasm almost completely filled with hemoglobin. Late forms red staining, eosinophilic. Nucleus chromatin condensed, dark, homogenous structureless mass referred to as pyknotic.

Reticulocytes

Slightly larger than normal erythrocyte. Nucleus has been extruded. Reticulum or filamentous substance, varying in amounts and arrangement, stains deep blue with brilliant cresyl blue; the smallest amounts seen in those nearly mature. Reticulum corresponds to basophilia of cytoplasm and is not related to nuclear remnants, mitochondria, or hemoglobin. In two to five days, perhaps in less time, reticulocytes mature in circulation and normally number 0.5 to 1.5 per cent. Reticulocytes reflect reactivity of the bone marrow; an increase indicates accelerated hematopoiesis. Reticulocytes show maximal peaks following specific treatment of patients with

tissues. The major physiologic method by which red cells are destroyed is by fragmentation caused by the traumatic effects of circulation. Erythrophagocytosis within the spleen and lytic factors present in the plasma and tissues are contributory agents in this physiologic process. Phagocytosis of intact red cells may be observed occasionally in the peripheral blood and bone marrow in patients with erythroblastosis and acute leukemia.

Although phagocytosis and hemolysis may be involved in this process these factors exert their influence largely in pathologic conditions. Fragmented cells (schistocytes) formed in this manner also result from the wearing out of enzyme systems controlling the integrity of the cell or its membrane. Stagnation in the spleen can also contribute to red cell dissolution by altering the spheroidicity and osmotic fragility.

The destruction of the red blood cell is accompanied by a breakdown of hemoglobin. Following preliminary oxidative opening of the protoporphyrin ring and the formation of a green bile pigment (iron protein complex) verdohemoglobin, iron is split off. Iron attached to a beta 1 globulin of the plasma (siderophyllin transferrin) is transported in the blood stream to the bone marrow for the regeneration of hemoglobin or to the liver, spleen and other organs where it is deposited in the form of ferritin and when excessive hemosiderin.

Formation of Bilirubin. Bilirubin is derived from hemoglobin. It is the globin and iron free fraction of the hemoglobin molecule. An increased destruction of red cells during hemolysis results in a corresponding increase in bile pigment production. When iron is split off the compound has been stated to exist for a time as a bilirubin globin complex. Following the loss of iron the green bile pigment is formed. The separation of globin and the degradation to bilirubin probably occur in the reticuloendothelial system. Globin enters the body protein pool as amino acids to be utilized in hemoglobin formation.

Bilirubin pigment is formed in the reticuloendothelial elements of the spleen, lymph nodes, bone marrow, liver and connective tissue (probably with the bone marrow as the most significant site). About 10 to 15 per cent of total bile pigment is derived from precursors other than hemoglobin^{21,22} such as heme not used in the synthesis of hemoglobin, myoglobins, peroxidase, catalase and cytochromes. Bile pigments in human serum range from 0.5 to 0.8 mg. per 100 ml. of blood and those in the serum of patients with chronic hemolytic anemias range from 1 to 3 mg., rarely above 5 mg. When normal bilirubin levels occur in a patient with hemolytic anemia it is due to the ability of a healthy liver to excrete excess quantities of the pigment. The accumulation of indirect bilirubin in the plasma as occurs in patients with erythroblastosis and in those in a hemolytic crisis results from overloading of the liver with the products of blood destruction.

London¹ includes the following considerations in evaluating the gross bile pigment metabolism: the amount of hemoglobin which is being destroyed daily; the functional capacity of the liver to excrete bilirubin into the bile; the patency of the biliary tree; the functional capacity of intestinal bacteria to reduce bilirubin to the urobilinogen; the functional capacity of the liver to re-excrete urobilinogen; and the threshold of the kidney for the excretion of direct bilirubin.

The multiple designations given to the same blood cells have led to an attempt to standardize the nomenclature and to provide more precise criteria for identity of each cell type.⁸ The recommended terms for the red cell series and a few of their alternates include the following: rubriblast (megaloblast, hemocytoblast), prorubricyte (pronormoblast), rubricyte (basophilic and polychromatic normoblasts), and metarubricyte (orthochromatic normoblast). "Pernicious anemia type" is applied as a qualifying phrase to any cell in this series in which the morphologic changes are those of pernicious anemia.

Normal Destruction of Erythrocytes The destruction of the red cell is accompanied by a number of phenomena of which those relative to the excretion of bile pigment are among the most important.

Life Span of the Red Blood Cell The life span of the human red cell in the circulation is approximately 120 days, corresponding to a normal rate of replacement of 0.83 per cent per day. The methods used for this determination are based on the Ashby method of differential agglutination⁹ in which the number of transfused group O red cells is determined periodically in the blood of the recipient of another group. The same objective can be achieved with the use of compatible blood for the major blood groups but with differences in the M and N factors. In either case the agglutination of the recipient's blood by appropriate sera permits the counting of the residual unagglutinated cells of the donor. Comparable results for the normal life span have been obtained with the use of red cells tagged with an isotope of nitrogen (N^{15}) or of iron (Fe^{59}) or of chromium (Cr^{51}).

Radioactive sodium chromate can be satisfactorily employed to measure the circulating red cell mass, blood volume, and red cell survival and to localize the site of red cell destruction.¹⁰⁻¹² The method of labeling red cells by radioactive chromium has replaced the other more elaborate methods. It affords a simple method for studying red cell survival in normal subjects and in patients with various hematologic disturbances. By reinjecting blood which had been mixed with the sodium radiochromate into the circulation of the patient, the longevity of the cells is measured in their natural environment. The chromium taken into the cell becomes firmly attached to the globin portion of the hemoglobin molecule.

The half-life survival of Cr^{51} tagged red cells has been found to average from 26 ± 2 days¹ to 33.1 ± 3.2 days.¹³ When correction is made for the loss of radioactivity by elution from the red cell of 1 per cent per day, the half-life of chromiumated cells corresponds to the half-life of transfused cells as determined by other methods. A considerably reduced half-life has been noted in many of the intrinsic hemolytic anemias or in blood dyscrasias accompanied by a hemolytic component.¹²

Fetal red blood cells obtained from the umbilical cord and tagged with Cr^{51} are noted to have a shortened life span with a half-life of 14 to 22 days when they are injected into normal individuals.¹⁴

Normal Hemolysis With a normal life span of 120 days, approximately 1 per cent of the red cells leave the circulation daily. Under normal conditions worn-out red cells are removed from the circulation by cells of the reticuloendothelial system in the spleen, bone marrow, and liver and to a lesser extent in other

filtered by the kidneys and that relatively insoluble indirect bilirubin is incapable of being filtered. Hence the indirect type of bilirubin does not appear in the urine despite excessive amounts in the blood. Increased excretion of bile by the liver however accounts for the excess of urobilinogen in the urine of patients with hemolytic anemia. On the other hand the soluble direct bilirubin can be excreted by the kidney when it accumulates in the blood as it does in patients with obstructive jaundice and hepatic or duct disease.

Urobilinogen Excretion—an Index of Hemolysis Bile pigments are derived from the breakdown of heme and the amount found is related almost quantitatively to the hemoglobin produced and destroyed each day. The porphyrin portion of hemoglobin exclusive of the iron constitutes 3.5 per cent by weight of the hemoglobin molecule. Theoretically all of the porphyrin of the destroyed hemoglobin is converted to bilirubin. Thus 1 gm of hemoglobin yields 35 mg of bilirubin.⁶ However 10 to 15 per cent more than the estimated quantity actually appears each day as urobilinogen.⁷⁻¹³ The precursors of this additional fraction are some porphyrin which is synthesized in the liver and not incorporated into red cells and porphyrin derived from the destruction of hemoproteins other than hemoglobin (myoglobin and cytochromes).

Bilirubin is excreted into the bile and passes into the colon where it is reduced by bacterial action to a group of urobilinogens consisting of mesobilirubinogen and stercobilinogen. Oxidation in the urine and stool results in conversion of colorless urobilinogen to the colored urobilin. A portion of the urobilinogens is excreted in the stools but in large part is reabsorbed from the intestine to re-enter the liver in the portal circulation and is then re-excreted in the bile. A few milligrams leave the general circulation to be excreted in the urine.

Quantitative estimates of urobilinogen excretion are an index of hemoglobin breakdown since the bulk of bile pigments stem from this source. It has been estimated that 1 mg of urobilinogen is derived from approximately 24 mg of destroyed hemoglobin. Increased hemoglobin destruction is invariably associated with excessive urobilinogen excretion in the feces and usually in the urine. The presence or absence of urobilinogen in the urine largely depends upon the capacity of the liver for re-excretion in the bile. With hepatic damage or dysfunction from overload re-excretion of urobilinogen is impaired a spill-over in the blood occurs and urobilinogen appears in the urine. The normal daily adult excretion of urobilinogen in the feces is 40 to 280 mg⁴ in the urine it is 0.5 to 3.5 gm. In infants and children the normal daily mean value for fecal urobilinogen ranges from 38 mg under 1 year of age to 45.2 mg from 10 to 14 years of age in patients with hemolytic disease it ranges from 15.1 mg to 205 mg respectively.³

Compensated Hemolytic Disease The processes of blood destruction and formation are normally balanced so that the concentration of hemoglobin and red cells in the circulating blood remains unaltered. In a state of balance the red cells and hemoglobin destroyed are replaced each day. When blood destruction is increased bone marrow activity is accelerated more red cells and hemoglobin are produced and equilibrium is re-established. Within limitations an excessive degree of hemolysis will not result in anemia in a patient with ade-

Direct and Indirect Bilirubin Two types of bilirubin have been differentiated on the basis of the van den Bergh reaction. The basis of this test is the observation by Ehrlich that the addition of diazo reagent (diazotized sulfanilic acid) to blood plasma or other bile containing solutions yields a reddish violet color. The immediate evolution of maximum color intensity is termed a direct reaction. If color fails to develop in one minute but appears after the addition of alcohol the reaction is regarded as indirect. No completely satisfactory explanation has been offered for the direct and indirect reactions although it is now known that the direct reaction depends upon the formation of soluble glucuronides of bilirubin.

The van den Bergh test employs these reactions to distinguish the two types of bilirubin involved in clinical jaundice. If the jaundice results from intrahepatic or extrahepatic obstruction to the passage of bile into the intestine most of the bilirubin is of the direct reacting form. This is the one minute bilirubin. When jaundice is due to excessive destruction of hemoglobin as in hemolytic anemia most of the bilirubin is in the indirect reacting form that is the addition of alcohol is essential for color to develop. It is possible that the alcohol catalyzes the transformation of indirect to direct bilirubin so that the diazo reagent will produce the reddish violet color. The total concentration of both types of bilirubin usually ranges from 0.2 to 1.4 mg per 100 ml with 0.2 mg as the upper limit of normal for the direct type.

Klatzkin and Bungards have shown that the bulk of bilirubin in serum is bound to albumin irrespective of its behavior in the van den Bergh reaction. They found no evidence that in the breakdown of hemoglobin globin remains attached to its porphyrin fraction to yield indirect reacting bilirubin. The action of alcohol in facilitating the diazotization of indirect reacting bilirubin does not depend upon release of bilirubin from its protein complex. This contradicts the former concept that the indirect reacting bilirubin is attached to alpha globulin in contrast to the linkage of direct reacting bilirubin to serum albumin.

It has also been postulated³ that the direct bilirubin is a bilirubin metal complex and that indirect bilirubin represents bilirubin not bound to metal.

The excretion of bile depends upon the conversion of indirect to direct bilirubin. This process has heretofore been regarded as a function of enzyme systems in the liver which are active in the dissociation of pigment protein complexes. It has recently been demonstrated however that the water insoluble indirect bilirubin requires conjugation with glucuronic acid for the formation of water soluble direct bilirubin which is then capable of excretion in the bile. The terms bilirubin and conjugated bilirubin have therefore been suggested as substitutes for "indirect bilirubin" and "direct bilirubin" respectively.⁴ According to this concept indirect bilirubin being water insoluble requires prior addition of alcohol to initiate coupling with the diazo reagent whereas conjugated bilirubin being water soluble is readily diazotized. The formation of bilirubin glucuronide is mediated by a glucuronyl transferase enzyme system and the delay in its formation accounts for the accumulation of indirect bilirubin in the plasma in jaundice of the new born infant (see Chapter 8). In obstructive jaundice conjugated (direct) bilirubin gains access to the blood and then to urine.^{3,41} It is thus understandable that soluble bilirubin glucuronides (direct reacting bilirubin) can be readily

filtered by the kidneys and that relatively insoluble indirect bilirubin is incapable of being filtered. Hence the indirect type of bilirubin does not appear in the urine despite excessive amounts in the blood. Increased excretion of bile by the liver however accounts for the excess of urobilinogen in the urine of patients with hemolytic anemia. On the other hand the soluble direct bilirubin can be excreted by the kidney when it accumulates in the blood as it does in patients with obstructive jaundice and hepatic or duct disease.

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quately compensating bone marrow activity. According to Crosby and Akeroyd¹⁰ hemolytic anemia follows only when the average life span of the red blood cell (normally 120 days) becomes so short that the bone marrow working at maximum capacity (6 to 8 times the normal) cannot maintain an adequate output of hemoglobin and red blood cells. They have emphasized the important fact that abnormal hemolysis without anemia is indicative of a completely compensated hemolytic process and that the underlying feature of the hemolytic syndrome is reduced survival of the red cells. When these limits are exceeded and anemia develops, equilibrium may be reestablished at lower levels depending upon the capacity of the bone marrow to form red cells and the extent of their survival in the circulation.

When the rate of red cell destruction exceeds the rate of red cell production the red cell volume is lowered and fewer red cells are being destroyed per unit of time. When the red cell volume has been reduced to that point at which the number of red cells being destroyed is equaled by the number of red cells being produced, a new equilibrium is established resulting in a stable but anemic hemoglobin level.¹¹ At any one time in a hemolytic disease a particular hemoglobin value is the resultant of the extent of the hemolytic defect present and the particular capacity of the bone marrow to respond with an increase in hemoglobin synthesis and red cell production.

The concept of compensated hemolytic disease is important in appraising blood conditions in which blood levels appear unduly high in the face of a known hemolytic process. Because of this mechanism, anemia in hereditary spherocytosis (familial hemolytic anemia) may not always be present, and in patients with the true or mild form of Mediterranean anemia overcompensation accounts for the polycythemia at times in association with elevated hemoglobin levels. Evidences of increased hemolysis can be detected with the aid of appropriate laboratory tests. A significant increase of reticulocytes represents a simple index of compensated hemolytic disease. In erythroblastosis fetalis augmented reticulocyte values with a normal red cell count on the first day of life may indicate increased red cell turnover and the need for close watch in succeeding days for the development of anemia and jaundice. In crises of sickle cell anemia the hemoglobin level often remains unchanged but increased reticulocytes reflect compensatory bone marrow activity.

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Erythrocytes—Morphologic Abnormalities

General Considerations Of all of the laboratory procedures the most important for its diagnostic value yet the simplest is the examination of the blood smear. Although corroborative evidence from auxiliary sources may be required to establish a final diagnosis the stained film constitutes a visual representation of the effect on morphology of the factors involved in the pathogenesis of a specific anemia. In describing the changes in the red blood cells it should be pointed out that there are few specific red cells that are indicative of a particular disorder. Target cells, oval cells, hypochromic microcytes and basophilic stippling and hypochromic microcytes appear in varying percentages in certain stages of many anemias and therefore cannot be regarded as distinguishing features of a single disease. They are significant with the support of other pertinent information.

Although overlapping occurs and differentiation from the normal is often difficult the following represent the most conspicuous alterations in size, shape and structural changes and staining peculiarities of the erythrocytes.

Abnormalities in Size Following are abnormalities of size which may be noted.

Anisocytosis The excessive variation in size of the red cells (average diameter 7.5 microns) is termed anisocytosis.

Poikilocytosis Marked irregularity in the shape of the red cell is termed poikilocytosis. The pear shaped cell is most commonly found in those patients with anemias in which there is marked variation in size of the cells.

Microcytes Microcytes are cells with diameters of less than 6.5 microns and usually characterize the blood smear of the patient with iron deficiency anemia. Microblasts or microcytic nucleated red cells are increased in the bone marrow of patients with iron deficiency or blood loss. Pointed projections may extend from the cytoplasm. The chromatin of the nucleus is condensed and deeply stained.

Macrocytes Macrocytes are found principally in patients in whom there is a deficiency of liver maturation principle such as in those with pernicious anemia or in patients with a deficiency of folic acid such as in megaloblastic anemia of infancy. They are large cells with a diameter of 8.5 microns or more, are thicker than the normocyte, are well filled with hemoglobin and possess a mean corpuscular volume greater than normal.

Hypochromic Macrocytes Hypochromic macrocytes are found in small num-

bers in the blood of patients with many of the chronic anemias of childhood. They appear in larger numbers in the blood of patients with Mediterranean anemia of varying grades of severity; they are of especial value in the diagnosis of the mild form. Normochromic macrocytes are observed in small numbers in the blood smear of the patient with aplastic anemia in childhood.

Spherocytes (Microspherocytes) In hereditary spherocytosis the spherical shape of the cells results from a developmental defect which occurs after the erythroblast stage and in which a normal cell volume is enclosed within a greatly diminished surface area. Spherocytes are globular, thick red cells of lessened diameter but usually are normal in volume; hence they are readily hemolyzed in hypotonic solutions of sodium chloride. Since normal red cells become thicker as they take up water, spherocytes already globular require less water before hemolysis sets in. Mechanical fragility is also increased. The small, deeply stained spherocytes which characterize congenital spherocytic anemia may also be observed in varying degrees in patients with other conditions associated with hemolysis, such as acquired hemolytic anemia and erythroblastosis due to sensitization by the A-B agglutinogens, and less frequently in patients with congenital nonspherocytic hemolytic anemia, leukemia, and conditions of stasis within the spleen and in stored blood. Only the peculiar shape of these cells renders them susceptible to trapping by the spleen.

Abnormalities in Shape Another morphologic abnormality of erythrocytes is irregularity of shape.

Elliptocytosis (Ovalocytosis) Oval or elliptical cells occur typically as a dominant hereditary anomaly in which they constitute from 25 to 90 per cent of all erythrocytes. The cells vary in shape from the rod or elongated forms to the oval shape, all being well filled with hemoglobin. Contrary to sickle cells, these cells cannot be made to sickle in an environment of lowered oxygen tension. Elliptocytosis is usually benign and symptomatic; however, asymptomatic mild compensated anemia and more rarely, overt hemolytic anemia have been reported as complications in about 15 per cent of the patients.¹⁴

The average survival of elliptocytes transfused into a normal recipient was found to be about 30 days⁹ or 60 days by the Ashby technique¹⁰ in contrast to the normal of 120 days. Measured by sodium chromate (Cr^{51}), the survival of the patient's red cells within his own circulation is considerably reduced, with a half life of 18.5 days as compared with the half life of normal cells of 33.1 days.¹² Nucleated red cell precursors show no abnormalities of shape. Elliptical cells occur in smaller numbers in the blood of patients with sickle cell disease,^{7, 9} and in that of the newborn infant.¹ They do not reach their maximum level in the infant until he is 3 to 4 months of age. On the other hand, ovalocytosis may be associated with hemolytic anemia and be accompanied by hyperbilirubinemia soon after birth; in such cases exchange transfusion should be considered.

Oval and elliptical cells are often noted in association with marked microcytosis and poikilocytosis. It is necessary therefore to differentiate symptomatic from hereditary elliptocytosis,¹ since oval shaped cells appear in variable numbers in the blood of patients with Mediterranean anemia, severe iron deficiency anemia, pernicious anemia, anemia of infection, leukemia, and hereditary nonspherocytic

hemolytic disease. Also included is a type of familial hypochromic microcytic anemia with splenomegaly affecting male members.

Sickle Cells (*Drepanocytes*) The characteristic cells are elongated and narrow with rounded pointed or filamentous ends. Although they appear in the stained smears of the severely anemic patient they are greatly increased in number in sealed wet films with reduced oxygen tension. Reversion to the normal form occurs with exposure to oxygen or carbon monoxide. Frequently in the trait or sickle anemia the cells assume a holly leaf appearance exhibiting numerous superficial spines. The mature orthochromatic normoblast can be made to sickle slowly although stained bone marrow smears show no morphologic changes in the nucleated red cells.

The sickling phenomenon is based on the presence of an abnormal form of hemoglobin which can be separated from normal hemoglobin by differences in electrophoretic mobility.¹⁸ This disparity between the two hemoglobins is caused by the globin rather than the heme part of the molecule. The peculiar shape of the erythrocytes has been attributed to the greatly diminished solubility of reduced sickle cell hemoglobin as compared with reduced normal hemoglobin. This results in intracellular crystallization of the hemoglobin¹⁹ and increased viscosity with tactoid formation resembling sickled erythrocytes.⁸ A minimal value of at least 20 per cent of sickle hemoglobin is required to elicit the sickling phenomenon.

Ovalocytosis has been observed in association with the sickle cell trait with evidence of both varieties of cells in the peripheral blood.⁹

Target Cells In target cells hemoglobin is concentrated in the periphery and in the center producing concentric light and dark zones after staining. The deeply stained central area gives the impression of a bull's eye in a target.¹ Target cells are a variety of leptocytes or thin cells whose cell envelope is too large and out of proportion to its meager contents and accounts for their increased osmotic resistance in hypotonic solutions of sodium chloride.

Target cells originally regarded as diagnostic of Mediterranean anemia are now known to be nonspecific and are found in increased numbers in many anemic states in infants and children in patients with obstructive jaundice and hypochromic anemia following splenectomy and especially in patients with conditions associated with abnormal hemoglobins. Target cells are present in patients with both mild and severe Mediterranean anemia (usually up to 10 per cent) are further increased in those with sickle cell anemia and appear in larger numbers in patients with a combination of these diseases (microdrepanocytic or thalassemia sickle cell disease). These cells are prominent also in the blood of patients with the hemolytic diseases associated with other pathologic hemoglobins. In those with sickle cell hemoglobin C disease for instance they are present to the extent of 40 to 85 per cent of all red cells.¹⁰ It has been suggested that large numbers of target cells in the blood smear indicate the presence of C hemoglobin. Whereas target cells are also increased in patients with hemoglobin E disease perhaps less than in those with C hemoglobin few or none of these cells has been noted in reported cases of sickle cell-hemoglobin D disease.⁷

Leptocytes Leptocytes are abnormally thin erythrocytes some are bowl

shaped and assume the appearance of the target cell following drying and preparation of the film. Leptocytosis refers to a preponderance of leptocytes such as occur in patients with hypochromic microcytic anemia and Mediterranean anemia. The term hereditary leptocytosis is an alternative name for the latter disease. Other cells found in the latter condition are nontarget cells but are extremely thin leaflike and of remarkable transparency.

Miscellaneous Changes Miscellaneous morphologic abnormalities in erythrocytes are considered in the following discussions.

Basophilic Stippling. Basophilic stippling or punctate basophilia already described applies to the presence of round fine or coarse bluish violet or dark blue granules scattered in the cytoplasm of the polychromatophilic red blood cells. They represent regeneration or immaturity of the cell and are sometimes best observed in slightly thicker and moderately overstained blood smears. They are associated with all chronic anemias, leukemia, iron deficiency anemia, the trait or mild form of Mediterranean anemia, and lead poisoning. They are to be differentiated from the network found in reticulocytes in cresyl blue preparations.

Hypochromia and Hyperchromia. Hypochromia is observed in cells with a lack of hemoglobin and is represented by an increase in central pallor. Deeply and homogeneously stained red cells lacking central pallor because of complete hemoglobinizations are termed hyperchromic. A macrocyte with a normal concentration of hemoglobin contains more of this substance than a normocyte only because of its size. Since oversaturation with hemoglobin does not occur, the cell cannot be truly regarded as hyperchromic. Nevertheless, according to common usage, hyperchromic anemias identify conditions in which macrocytes prevail and in which the color index is greater than unity.

Siderocytes. Siderocytes are red blood cells containing nonhemoglobin iron particles which are visualized by the Prussian blue method of staining. Sideroblasts are normoblasts containing similar iron inclusions. Both types of cells in the peripheral blood and bone marrow, respectively, appear in moderate numbers in normal persons. They are increased in those with infection, aplastic and hemolytic anemias and are markedly decreased in those with iron deficiency anemias.¹ Siderocytes are significantly increased after splenectomy.

Heinz (Heinz Ehrlich) Bodies. Heinz bodies are moderately sized round or irregularly shaped protein containing granules lying at or close to the periphery of the red blood cell. They are single or multiple refractile inclusion bodies and are observed after supravital staining with brilliant cresyl blue and methyl violet. The Romanowsky dyes such as Wright's stain obscure their presence. They are easily detected as refractile bodies in unstained wet preparations. These intracellular inclusions have been described as refractile agglomerations of denatured globin, verdohemoglobin, and perhaps stromal material. Heinz bodies represent rather severe intoxication of the red cell² and are particularly noticeable in the presence of intrinsic defects within the cell. They are to be differentiated from Howell-Jolly bodies, reticulocytes, and siderotic granules.

The presence of Heinz bodies in significant numbers is indicative of injury to the red cells and serves as an index of existing or impending anemia. Sulfo-namides, primaquine, phenylhydrazine, naphthalene, phenacetin, and the fava

be associated with red cell destruction are prominent among Heinz forming compounds.⁶ They appear in increased numbers in patients with hemolytic anemias and leukemia following splenectomy,⁴ and in patients with agenesis of the spleen. (See Chapters 8 and 15.)

Howell Jolly Bodies Howell Jolly bodies are small rounded densely staining nuclear remnants occurring singly or doubly and are eccentrically placed in the red cell. They stain a reddish blue or dark violet with Wright's stain and are prominent after splenectomy and in many anemias such as severe iron-deficiency anemia, pernicious anemia, spherocytic anemia and leukemia. The origin of these bodies has been ascribed to abnormal mitosis in the late megaloblast stage when single chromosomes or groups of chromosomes become detached, fail to be included in the formation of the interphase nucleus and remain free in the cytoplasm as nuclear remnants.¹¹

The presence of target cells, siderocytes, normoblasts and Howell Jolly and Heinz bodies in varying combinations in the peripheral blood of a young infant prompts consideration of agenesis of the spleen, especially when associated with serious anomalies of the heart and situs inversus of the abdominal viscera.⁴ It has been stated that the diagnosis of asplenia can be made with confidence in infants with congenital cardiac disease who are polycythemic and whose peripheral blood shows numerous Howell Jolly bodies.¹

Cabot Rings Cabot rings are basophilic rings, circular or twisted into a figure of eight which occur occasionally in red blood cells of patients with hemolytic anemias, untreated pernicious anemia, leukemia and lead poisoning. They stain reddish purple with Wright's stain. Originally regarded as nuclear remnants, they are now interpreted as an expression of cellular degeneration resulting from the action of hemolytic agents.¹

Crenation In blood smears which dry slowly the red cell envelope becomes exposed to a hypertonic medium which causes wrinkling of the surface with the appearance of a moderate number of knoblike or prickly projections. This process known as crenation occurs normally. Two other types of crenated cells have been described which, however, are of pathologic significance.

Burr Cells The burr cell is a mature red cell possessing one to several spiny projections along its periphery and represents a type of preformed poikilocyte. Burr cells have been especially noted in persons with conditions with impaired renal function.¹ They appear in infants in connection with toxic hemolytic anemia associated with Heinz body function. In the first trimester of life they appear in small numbers in normal full term and premature infants (approximately 2 to 6 per cent maximally, respectively) and increase transiently up to 50 per cent in patients with severe hemolytic anemia.⁹ Burr cells may, however, appear sporadically beyond this age in patients with severe hemolytic disorders.

Acanthrocytes Acanthrocytes (thorny red cells) resemble the burr cell but in the stained film look like spherocytes with pseudopods. The projections are large, coarse and irregularly spaced. These malformed cells were observed in large numbers in the blood of a child with progressive atoxic neuropathy.⁶ The differentiation between acanthocytes and burr cells may be difficult and bizarre.

erythrocytes of an intermediate type have been observed as an inherited abnormality in association with retinal pigmentary degeneration

Familial Erythroid Multinuclearity A familial erythroid anomaly has been observed²⁰ in which giant sized erythrocytes giant plurinuclear red cell precursors and nucleated red cells with coarse cytoplasmic stippling and karyorrhexis have been found in the bone marrow. Although persons with this anomaly have no symptoms they have a tendency toward mild anemia with peripheral blood showing anisocytosis with a tendency toward macrocytosis and poikilocytosis

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Blood Groups

Blood Group Factors Blood group factors are genetically determined substances on the surfaces of erythrocytes which are present in certain persons and absent in others. The blood group factor is antigenic and is that portion of the red cell which combines with a specific antibody. Of the blood systems the ABO and Rh groups are most important clinically.

Naturally occurring antibodies in significant concentration are normally absent in the Rh group and numerous minor blood types and are present in the ABO groups. Both the ABO and Rh factors are antigenic and if they are introduced into a host in whom they are absent antibody is formed. Each factor is highly specific and can be differentiated by its behavior with the corresponding antibody. If cells containing one of these antigens are suspended in serum containing the corresponding antibody the antibody unites with the antigen with resulting agglutination of the red cells which may then undergo lysis. The significance of the blood group factors stems from hemolytic reactions when red cells are transfused into recipients with corresponding antibodies within the circulation and during pregnancy when antibodies of maternal origin cross the placenta destroy ing fetal red cells. Thus the blood group systems ABO and Rh are of paramount importance in the pathogenesis of hemolytic transfusion reactions and in erythroblastosis.

Definitions of Terms in Relation to Blood Groups Several terms are frequently employed in the various relationships of the blood groups. They may be defined as follows:

immunization refers to the formation of antibodies when erythrocytes containing specific blood group factors are injected into individuals of the same species who lack this factor.

heteroimmunization refers to immunization by injection of red cells into members of another species lacking this factor.

blood group typing refers to the determination of blood groups with the use of specific antisera. In transfusions the importance of cross matching is to prevent the reaction of the donor's red cells to the serum of the recipient.

cross matching test for compatibility between the patient's serum and the donor's red cells. This is necessary even when the patient and donor belong to the same blood group because of the presence of subgroups and rare types.

alleles or allelomorphs alternative forms of genes similar in type but not identical which

- occupy the same position on a pair of corresponding chromosomes each chromosome contains a series of places or loci occupied by different genes
- homozygous* refers to individuals having identical allelic genes on the two corresponding loci of a pair of chromosomes
- heterozygous* having two different allelic genes on the two corresponding loci of a pair of chromosomes
- titer* a measure of the relative amount of antibody determined by measuring the highest dilution of serum which can visibly agglutinate cells (in this context erythrocytes)
- heterospecific pregnancy* a pregnancy in which the fetal red cells possess an A or B factor not present in the mother's cells. Also the mother's blood contains an anti A or an anti B agglutinin which is incompatible with the A and B agglutinogen in the red blood corpuscles of the fetus
- phenotype* the characteristics of an individual represented by direct observation and not revealing all the separate genes responsible for these characteristics
- genotype* refers to the genetic constitution of an individual for a particular characteristic (in this instance blood groups)
- propositus (proband)* the original member of the family whose blood group (or other presenting physical or mental characteristic) prompted a genetic or hereditary study

ABO Blood Group System It has been established that the blood of any person falls into one or another of the four well defined major groups according to the antigens in their red cells. The presence or absence of each of these blood group factors is determined by their agglutinating reaction with high titered anti A or anti B serum. The validity of these tests may be confirmed by adding inactivated serum from the subject to a suspension of known A and B cells. Anti A and anti B are almost invariably present in the blood when the corresponding antigen is absent.

Distribution of ABO Groups In the United States the incidence of each group is as follows: group O 45 per cent, A 41 per cent, B 10 per cent and AB 4 per cent.

Inheritance of ABO Blood Groups Inheritance of ABO groups depends upon three genes A, B and O. Since an individual inherits the A, B or O factor from each parent, the combination of genes in the body cells determines the genetic components in the body cells: AA, AO, BB, BO, AB and OO. The A gene occurs in two forms, A_1 and A_2 , the former being dominant and more frequent. Thus about 80 per cent of the persons belonging to group A are included in subgroup A_1 and about 20 per cent in subgroup A_2 . With anti A and anti B typing serums the four phenotypes A, B, AB and O may be detected as shown in Table 1.

Table 1 Reactions With Anti A and Anti B Serum

Blood Group	Anti A Serum	Anti B Serum	Serum Isoantibodies (Isoagglutinins)
O	—	—	Anti A, Anti B
A	+	—	Anti B
B	—	+	Anti A
AB	+	+	

+ = Agglutination

— = No agglutination

However the blood of persons with genotype AA and that of those with genotype AO cannot be differentiated from each other nor can the blood of persons with genotype BB be differentiated from that of those with genotype BO

Agglutinogens A and B are dominants and O is recessive. Several genetic and medicolegal principles stem from the ABO groups. For A or B to be inherited at least one parent must transmit either an A or a B gene to the offspring. It is apparent that a child cannot belong to group O if either parent belongs to AB for at least one dominant gene A or B must be received from the parent. Nor can a child be of any other group but O (genotype OO) if both parents are of that group. Also a parent belonging to group O cannot have a child who belongs to group AB.

H Substance in Red Cells It is not unusual for O cells to be designated as O (H). H refers to a basic substance from which the A and B substances are made under the influence of the A and B genes. H is present in largest quantity in group O red cells and least in A and B red cells.¹⁶ A large number of reagents of human animal and even plant origin (such as extracts of *Ulex* seeds) have been discovered that react with group O cells but not with cells belonging to group A and B. It is not possible, however, to distinguish homozygous AA and BB cells from heterozygous AO and BO cells by titration against the corresponding A or B isoagglutinins.

Development of Blood Groups A and B isoagglutinogens can be demonstrated in the fetus in the second and third months of life.¹³ Anti A and anti B isoagglutinins found in the cord blood diminish in titer or completely disappear in the first weeks or months of life. That these isoagglutinins have been passively transferred from the mother is evident from the similarity existing between the blood of the newborn infant and that of the mother with respect to isoagglutinins.¹⁹ The isoagglutinins in the newborn infant's serum are therefore not its own but are derived from the mother by filtration through the placenta. In many pregnancies, however, the placenta exerts a definite limiting effect upon the extent to which the maternal agglutinins can enter the baby's circulation.⁹ Between 3 and 6 months of age the child begins to make its own isoagglutinins. The strength of the isoagglutinins rapidly rises and is at its maximum at 5 to 10 years of age; after this there is a gradual fall.¹⁷ The anti A titers are higher than anti B.

Secretors and Nonsecretors The blood factors A, B, and O are not restricted to the red cells since in approximately 80 per cent of human beings they are also present in water soluble form in the body fluids and in such secretions as saliva, gastric juice, nasal secretion, semen, and amniotic fluid.³ Persons who have blood group substances present in their secretions are known as "secretors" and those who do not are known as "nonsecretors." The blood group substances (agglutinogens) A and B are stable polysaccharide-amino acid complexes containing galactose and an amino sugar, N-acetyl glucosamine. The classification of a person as a secretor or a nonsecretor is most readily accomplished by testing the saliva where blood group substances are present in high concentration. It has been shown that practically all nonsecretors of A, B, and H substances are also positive for the Lewis (Le) blood factor. (See discussion of the Lewis group later in this chapter.)

Universal Donor and Recipient Since the red cells of group O are not agglutinated by any serum the persons belonging to this group are termed universal donors. However some group O blood may contain such a high titer of anti A and anti B agglutinins that despite dilution in the recipient's blood the titer may remain sufficiently elevated to cause serious red cell destruction. Generally such blood is considered safe if the titer is not over 1 to 100. Blood group specific substances A and B (Witebsky substance) are used to neutralize the anti A and anti B isoagglutinins of group O blood before it is given to persons of other blood groups. One unit (vial) is sufficient to reduce the anti A and anti B in 500 ml of blood to at least one fourth of its original titer.⁴ When group AB blood is not available for AB recipients experience has shown that lower titer group O blood is satisfactory. It has been estimated that the serum of 3 per cent of persons belonging to group AB contains anti O.

Rh Hr Blood Group System The discovery of the Rh factor provided an explanation for the cause of transfusion reactions other than those due to the four major blood groups of the ABO system described by Landsteiner. In 1940 Landsteiner and Wiener⁵ discovered the Rh factor. They found that when the blood of the rhesus monkey was injected into a rabbit the serum contained an antibody which not only agglutinated rhesus but human red cells as well. This factor corresponded in activity to one postulated previously by Levine and Stetson¹ to explain the cause of a severe reaction to the initial blood transfusion in a woman recently after delivery of her baby.

Further investigation revealed that the Rh factor was present in 85 per cent of the white population. People having this agglutino-gen were designated Rh positive whereas those in whom it was absent were designated Rh negative. In the Negro population the percentage of Rh positive is 93 per cent and in the Chinese it is 99 per cent. There are no naturally occurring antibodies to the Rh factor in contrast to their presence in the A and B groups. The Rh agglutinins may be produced however when an Rh negative person receives Rh positive blood.

A basic group of three sets of alternative antigens has been described within the Rh system. The capacity of a red cell to react with a given antiserum is based on the presence of a specific antigen in the cell. When one of the Rh antigens cannot be demonstrated as indicated by a negative reaction with its antiserum another contrasting or reciprocal antigen (Hr) is present in its place. Thus every person inherits one set of three Rh genes or corresponding Hr genes from each parent.

C D E Notations for Blood Groups The symbols C c D d E and e are used for the blood antigens detected by appropriate antiserum; the small letters corresponding to the Hr factors. Thus there are two sets of terminologies in relation to the Rh Hr system. An allelomorphic relationship exists therefore between rh and hr' Rh and Hr rh and hr' (C and c D and d E and e respectively).

Genetics of the Rh Hr Blood Types According to Wiener⁴ single Rh Hr genes with a series of alleles at one locus on the chromosome determine the presence of these factors in the blood. According to the Fisher Race concept¹⁴ on the other

Table 2 Six Basic Rh Hr Factors as Determined by Their Corresponding Antisera

<i>Wiener System</i>	<i>Fisher Race System</i>
rh	C
Rh	D
rh	E
hr	e
Hr	d
hr'	e

and each Rh blood factor is controlled by an individual gene in a series of pairs of alleles each at one of three adjacent closely linked loci on one of the twenty-four human chromosomes²

The six basic Rh Hr factors as determined by their corresponding antisera may be listed as shown in Table 2

Since the Rh Hr genes are alleles occupying the same locus or position of corresponding chromosomes a person must have an Rh antigen or one Hr antigen or both. Thus Rh testing with specific serums may reveal that the person has rh rh (homozygous) or rh hr (heterozygous). A person who is Rh negative must therefore be cde/cde. Rh positive persons on the other hand may possess a wide variety of genotypes the more common being CDe/cde CDe/CDe CDe/cDE and cDE/cde.

Using the Fisher Race nomenclature for the gene D or its allele d there are three possible genotypes DD Dd and dd. If anti d serum were available all three could be recognized. Since only anti D is available no distinction can be made between DD and Dd and both are similarly agglutinated.

There are three principal Rh factors Rh present in approximately 85 per cent of Caucasian people rh present in 70 per cent and hr present in 30 per cent. The symbol Rh has a capital R to indicate its special serologic and genetic position whereas rh and hr have a small r to indicate their lesser importance.

*Table 3 The Eight Standard Rh Allelic Genes**

<i>Gene</i>	<i>Corresponding Agglutinin</i>	<i>Blood Factors Present</i>
r	rh	hr' and hr
r	rh	rh and hr
r'	rh	rh' and hr'
r	rh	rh and rh
R	Rh	Rh hr' and hr'
R	Rh	Rh rh and hr
R	Rh	Rh rh and hr'
R	Rh	Rh rh and rh

clinically. For some reason the Rh₀ factor is a more potent producer of antibodies than are rh⁻ rh⁺ hr⁻ hr⁺ or Hr₀. The rh negative person is represented by rh since the cells do not react with anti Rh₀, anti rh⁻ and anti rh⁺ serums. Except for anti d (Hr⁻) testing serums are available for each of the Rh Hr factors. Despite this deficiency it is possible to hazard the presence of homozygosity or heterozygosity in a person on the basis of the statistical frequency of the reaction of the subject's cells to the available antisera.

For routine clinical use it is sufficient to subdivide human beings into Rh positive and Rh negative according to their reactions with the single anti Rh (anti D) serum. By this means it is found that of all of those who become immunized to Rh whether by transfusion or pregnancy approximately 99 per cent are Rh negative.¹⁶

The Rh Hr system receives its widest clinical application in erythroblastosis fetalis. Rh (D) is the most antigenic factor and is involved in approximately 93 per cent of cases of this disease.

In addition to the ABO and Rh Hr blood group systems there are other red cell antigens of clinical importance such as Kell and Duffy. Still others (MN, Ss, P, Lutheran and Lewis) are rarely if ever involved in human disease but are employed in precise identification of the red cell.

MN, Ss and P Blood Group Systems The MN, Ss and P blood group systems are rarely involved in intra group incompatibilities. In addition to their use in the investigation of the cause of obscure transfusion reactions they provide useful tools in human genetics and with the major blood groups help to resolve medicolegal problems of identity and parentage.

The M, N and P agglutinogens were discovered by Landsteiner and Levine⁸ who used the serums of rabbits immunized with human red cells. After absorption of species agglutinins the serums agglutinated some samples of human blood but not others. Naturally occurring antibodies against M and N are infrequent in the human being so that their detection requires the use of rabbit immune serums. Like A and B antigens M and N are well established in the newborn infant. Three genotypes occur MM, MN and NN. Type MN is found in 50 per cent of human beings, M in 30 per cent and N in about 20 per cent. A few human beings with acquired anti M antibodies have been encountered but these rarely lead to transfusion reactions or erythroblastosis. N is a much weaker antigen and has not been responsible for any difficulties.

Blood cells possessing the P factor are designated P+ and those lacking it P-. Naturally occurring anti P agglutinins can be found in a few unselected P negative persons. Anti P immunization is not known to cause erythroblastosis and rarely causes hemolytic transfusion reactions.

The antigenic factor S⁺ and related antigens are also present in human blood. Cases of erythroblastosis fetalis and hemolytic transfusion reactions have resulted from antigenic stimulation by anti S⁺. S⁺ bears a close genetic relationship to M and N¹⁷ and is probably closely linked with them on a single chromosome. A number of examples of anti S antibodies occurring spontaneously have been reported but more commonly they result from immunization.

Rh Variants Rh variants include the D factor C factor Kell groups (Kk) Duffy group (Fy^a and Fy^b) Lewis group (Le^a and Le^b) Lutheran group (Lu and Lu^b) Kidd group (Jk^a and Jk^b) factor U and low incidence antigens

D Factor D is a modified D (Rh_o) antigen which reacts weakly or not at all when tested with some anti D (Rh_o) serum. It is for this reason that such blood is occasionally classified as Rh negative. Its positive Rh character can be recognized by its reaction to high titered anti D serum and the indirect Coombs test. In the person with the D⁺ factor exposure of the blood to immune (incomplete) anti D serum will result in agglutination with antiglobulin (Coombs) serum. To avoid overlooking the D⁺ variant all blood designated as Rh negative should be subjected to additional screening by the indirect Coombs test.

C^w Factor The Rh antigen C^w (rh^w) is a third allelomorph at the C locus. Anti C^w antibodies result from transfusion with C^w blood and from isoimmunization in pregnancy¹⁵ causing erythroblastosis fetalis.

Kell Groups (Kk) The Kell, Duffy, Lewis, Lutheran and Kidd factors derive their names from the patients involved in cases of erythroblastosis fetalis and transfusion reactions and from prospective donors.

The Kell (K) and Cellano (k) factors are alleles with equal dominance. The Kell antibody has been responsible for many cases of transfusion reactions and erythroblastosis. In these respects it is secondary in importance only to the antibodies against D (Rh), A and B. On the other hand the Cellano (k) factor which is the reciprocal of Kell is of minor importance. No naturally occurring Kell antibodies have been described.

Duffy Group (Fy^a and Fy^b) For the Duffy, Lewis, Kidd and Lutheran systems a combination of two letters is used to indicate the blood group system and the particular gene or antigen is further designated by adding a b etc as a superscript¹⁶.

The Duffy system (Fy) has been shown to be responsible for hemolytic transfusion reactions but not as yet for erythroblastosis fetalis. Fy^a and Fy^b constitute two of the better known alleles of this factor. Anti Fy^b antibody is by far the more common of the two.

Lewis Group (Le^a and Le^b) Only a few cases of hemolytic transfusion reactions have been caused by anti Le^a . It is of interest that practically all persons whose red cells are Lewis positive are also salivary nonsecretors of AB and H substances and vice versa. Thus anti Le^a serum can be used to detect the nonsecretor of group A, B and O.¹⁶ Rosenfield and Ohno (cited by Mollison¹⁶) have also pointed out that the Lewis phenotype appears as an agglutinable characteristic of the red cells only after a few weeks of life, possibly because the Le^a and Le^b factors are absent or extremely weak at birth.

Lutheran Group (Lu and Lu^b) The Lutheran blood group system occurring in 6 per cent of the English population has no clinical importance. The Lutheran antibody has been detected in Lutheran negative subjects who have received multiple transfusions. This group plays no part in hemolytic transfusion reactions or erythroblastosis.

Kidd Group (Jk^a and Jk^b) Anti Jk^a was initially described in the blood of a patient who had given birth to an infant with erythroblastosis.¹ This patient had

never had a transfusion. There is as yet no conclusive evidence that this antibody is responsible for hemolytic transfusion reactions. The allelomorph Jk^b discovered later occurs even more rarely. The antibodies of the Kidd group are usually detected by the indirect Coombs test but preliminary application of trypsin to the red cells may be necessary.

Factor U The U factor was originally described in a patient with a fatal transfusion reaction.¹ This factor is almost universally present in human erythrocytes. The few persons who are negative to this factor possess blood of type N or MN.² Sensitization to the U factor is extremely rare and it is of interest that all clinical cases have been reported in Negroes. Of Negroes tested 12 per cent are U negative hence the possibility of sensitization by transfusion.¹⁴

Low Incidence Antigens ("Private Blood Group Antigens") Infrequent cases of intragroup immunization to a variety of rare antigens constitute a miscellaneous group known as "family antigens" or "private" blood group antigens.¹⁰ Here the positive or negative reactions are linked solely to members of a particular family. They are usually discovered in the investigation of cases of erythroblastosis fetalis in which the etiology is not accounted for by established blood groups. Of special interest is the Jy antigen (T_j). In five married women of child bearing age each of eighteen pregnancies ended in a miscarriage at two to five months gestation¹¹ because of incompatibility within this system.

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*Transfusions in Pediatric Practice**

Significant Factors in Transfusion Therapy The approach to transfusion in the pediatric patient necessitates certain background data in order to evaluate the need of transfusion in relation to the growth period potential risks and technicalities of administration. Such data summarized as follows also provide a basis for the indications and certain of the limitations of transfusion therapy.

Blood volume—80 ml per kilogram

Plasma volume—45 ml per kilogram

Erythrocyte volume—35 ml per kilogram

Hemoglobin concentration—12 to 13 gm per 100 ml

Hematocrit—36 per cent

1 gm hemoglobin = 3.4 mg iron

100 ml transfused whole blood (average)—30 mg iron

500 ml transfused whole blood (average)—250 mg iron

Dosage for transfusion

Whole blood

20 ml per kilogram when body weight is less than 25 kg

Units of 500 ml when body weight is more than 25 kg

Packed or sedimented erythrocytes

15 ml per kilogram when body weight is less than 20 kg

Units of 300 ml when body weight is more than 20 kg

Plasma—10 to 15 ml per kilogram

Indications for Transfusion The indications for transfusion are listed as follows:

To restore deficit of hemoglobin and erythrocytes

Acute and chronic blood loss

Hemolytic anemias—congenital and acquired

Defective blood formation

Pre- and postoperative

To restore blood volume

Shock, burns, trauma

To supply specific coagulation factors including platelets

*Quoted in part from Smith, C. H.: *Transfusions in Pediatric Practice*. Indications and Limitations. *Pediatrics* 17:598, 1956.

Miscellaneous

Infections

Hypoproteinemia

Exchange in erythroblastosis

Ionizing

In pediatric practice the indications usually apply to treatment of patients with blood disorders infection and nutritional and deficiency states. In contrast to the situation in the adult the management of transfusion needs in the child requires the additional appraisal of abnormality in relation to a specific stage of growth and development. For instance prescribing a hemoglobin level to be achieved by transfusion may not be a simple task in the infant or young child because the optimal value is not fixed but is subject to individual interpretation.

Blood Volume—Plasma, Total Circulating Hemoglobin, and Erythrocyte Mass. A knowledge of the shifts in blood volume has become almost mandatory in the interpretation of many clinical syndromes and in providing clues for their treatment. The need for recognizing fluctuations of blood volume (plasma total circulating hemoglobin and erythrocyte mass) was realized early in patients in surgical shock so that adequate physiologic restitution could be planned. Acute hemorrhage associated with blood disorders or accompanying shock burns or organic lesions calls for transfusion of calculated amounts of whole blood red cell suspensions or plasma when feasible. Blood volume estimations are now used in the treatment of patients with erythroblastosis in whom an exchange of at least twice the estimated volume represents current practice.

Comparisons in terms of total hemoglobin and erythrocyte mass permit evaluation of therapeutic procedures or the need for such procedures by eliminating the influence of variations in plasma volume. The determination of total circulating hemoglobin and erythrocyte content therefore has come into greater use because it frequently obviates erroneous conclusions drawn from a peripheral blood sample. From the blood volume (80 ml per kilogram of body weight) and hemoglobin measurements (expressed as grams per 100 ml) the total circulating hemoglobin mass can be readily determined. In patients in whom transfusions are being considered periodic estimations of the absolute hemoglobin mass frequently prove more valuable in detecting the early regeneration of hemoglobin than in determining the peripheral hemoglobin concentration. This calculation which takes into account the factors of growth and hemodilution has proved helpful in recognizing the initial states of hemoglobin regeneration in the premature infant¹⁵ and in evaluating the hemoglobin level prior to transfusion in the patient with refractory chronic hemolytic anemia such as Mediterranean anemia.¹⁶ In erythroblastosis for instance transfusions can be postponed in patients with hemoglobin concentrations of 8 gm per 100 ml at the fourth to sixth week of life in the so-called convalescent anemic phase¹⁶ when it is found that the total circulating hemoglobin mass either has stabilized or has begun to rise.

With certain exceptions a blood volume of 80 ml per kilogram of body weight

applies throughout life. In the newborn infant the value of 85 ml per kilogram has been found more accurate in calculating the blood volume prior to administration of exchange transfusion. In our experience the blood volume has been found to exceed the normal with values as high as 104 ml per kilogram in a small number of patients with sickle cell anemia who were not in crisis. An inordinate increase in plasma volume was responsible for such values. This hemodilution probably accounted for the freedom from disability of these patients since their hemoglobin values were maintained without transfusions at levels of 6 to 7 gm per 100 ml. In our experience crisis is associated with a sharp decrease in plasma volume but the amount of hemoglobin undergoes little or no change. In an attempt to restore the expanded precritical plasma volume whole blood and plasma have been given with moderate success. Increased fluid intake either orally or parenterally is a valuable adjunct.

Hemoglobin and Hematocrit Levels in Acute and Chronic Anemias In the management of severe anemia the objective in the restoration of a normal level of hemoglobin is different in acute blood loss than in chronic anemia. In the patient with nonrecurring hemorrhage or in the infant with severe iron deficiency who is too ill to wait the effects of specific therapy a sufficient amount of whole blood or preferably packed erythrocytes may be given to achieve hemoglobin levels of 12 to 13 gm per 100 ml and a hematocrit of 36 per cent.

The transfusion of packed erythrocytes to the patient with chronic anemia is to be contrasted with the need for rapid transfusion of whole blood to the patient with acute hemorrhage. Provided the cause of acute hemorrhage is corrected the bone marrow will be adequate to maintain normal blood levels subsequently. In patients with chronic hemorrhagic disorders completely normal levels of hemoglobin need not be attained with each transfusion if large amounts of blood are required to accomplish this end. A normally functioning bone marrow will eventually raise blood levels to desired values.

The hemoglobin value at which a transfusion is indicated in patients with anemias of the aplastic hypoplastic group or with chronic hemolytic anemias such as severe Mediterranean anemia and sickle cell anemia cannot be arbitrarily fixed rather it varies with associated signs and symptoms of the individual patient. As a guide to management patients with the aplastic hypoplastic and chronic hemolytic anemias usually do not require transfusions until hemoglobin levels decrease to 7 to 7.5 gm per 100 ml at which point clinical symptoms usually appear. Whereas blood levels in children with the aplastic hypoplastic anemias and in those with severe Mediterranean anemia tend to decline progressively unless treatment is given the patient with sickle cell anemia reacts differently. In the latter the need for repeated transfusions is much less urgent because hemoglobin levels usually stabilize in a range between 6 and 7 gm per 100 ml without discomfort or interference with activities and regardless of treatment. Although these criteria find general application the amounts of blood given and the intervals between transfusions require specialized study for each patient.

Choice and Dosage of Whole Blood Packed Erythrocytes and Plasma Although the choice and dosage of whole blood packed erythrocytes and plasma

are frequently clear cut they often depend upon the appraisal of factors other than the hemoglobin level and the erythrocyte count. A large body of experience has demonstrated the superiority of whole blood especially when blood volume and anemia require simultaneous correction. In patients with disorders of the clotting mechanism fresh whole blood is of value in supplying both the appropriate plasma factors and the erythrocytes lost by hemorrhage. Whole blood plasma or a combination play a part in the treatment of dehydration diarrhea and protein deficiency. The immunologic support represented by the transfer of circulating antibodies and gamma globulin in combating infection has now been replaced by the antibiotics and when needed by the gamma globulin fraction directly.

The administration of packed erythrocytes by permitting a larger dose in a single transfusion represents an ideal treatment in infants in patients with cardiac failure and in patients with chronic anemias refractory to other forms of therapy. To obtain concentrated suspensions of red cells plasma is removed from sedimented cells or after centrifugation. However in bleeding episodes associated with secondary thrombocytopenia due to bone marrow depression such as occur in patients with aplastic anemia and leukemia especially during chemotherapy fresh whole blood is preferable especially for its platelet content and possibly other constituents of plasma. Although the transfer of platelets may not be so effective as when concentrated platelet suspension is used¹ fresh whole blood is more easily obtainable and frequently controls hemorrhage satisfactorily.

Fresh platelet rich plasma collected in plastic bags and administered directly after bleeding the donor has proved to be one of the most effective means of controlling hemorrhage in patients with primary or secondary thrombocytopenia. Blood platelets drawn in this manner have a survival span of 4 to 8 days. For the actively bleeding patient with leukemia the continuous transfusion of platelet rich plasma and fresh whole blood checked by repeated hemoglobin determinations has been found to be one of the best methods of controlling severe epistaxis and bleeding from the gastrointestinal tract. When available platelet rich blood from a polycythemic donor given by direct transfusion through silicone coated syringes is ideal treatment for restoring both intact platelets and red blood cells.¹ Adrenocorticotrophic hormone (ACTH) and steroids (prednisone prednisolone and hydrocortisone) given intravenously also serve as valuable hemostatic agents. This form of therapy is especially indicated in patients with idiopathic thrombocytopenic purpura in the active state in patients being prepared for splenectomy and also in controlling hemorrhage in patients with secondary thrombopenic states.

Plasma transfusions restore blood volume when whole blood is not available correct a variety of clotting deficiencies (see Chapter 26) and supply protein in patients with hypoproteinemia. In treating patients with classical hemophilia individual units of fresh frozen group specific plasma are superior to pooled plasma in diminishing the risk of hepatitis.¹

In regulating the amounts of blood for transfusion it should be remembered that citrated (ACD) blood usually has a hematocrit of 32 to 36 per cent as

compared with 40 to 50 per cent for whole blood and 60 to 70 per cent for packed erythrocytes depending upon the amount of supernatant plasma removed in the latter ¹⁴

The dosages listed on p 75 serve as guides for the amount of blood and plasma to be given in a single transfusion In the case of whole blood and packed erythrocytes the values are calculated to elevate the hemoglobin concentration and erythrocyte count in varying degree depending upon the severity of the anemia but within safe limits of blood volume

With increasing weight and expanding blood volume these figures lose their significance and other criteria are substituted depending upon the objectives of therapy such as hemoglobin elevation cessation of hemorrhage and clinical improvement It should be obvious that the older child with chronic anemia who at irregular periods receives a single transfusion of 500 ml of whole blood and lesser amounts of packed erythrocytes cannot be expected to show a significant rise in hemoglobin The amount of blood is necessarily diluted in a higher blood volume than it is in the smaller child to whom similar amounts may be given Hence in determining the rate of survival of donor blood several transfusions are necessary within a short period of time to raise blood values to normal levels from which the rate of decline may be estimated

Transfusions in Premature Infants In most clinics a conservative policy is followed with regard to transfusions in the healthy premature infant At the age of 4 to 7 weeks when the infant's fetal and adult hemoglobin values have decreased to low levels spontaneous recovery usually begins with the predominant synthesis of the adult type ¹⁵ Administration of transfusions at a time when normal regeneration of hemoglobin is at hand seems unnecessary Besides the known complications to give transfusions at this point is to run the risk of depressing inherent bone marrow function ^{2,16}

The present trend is disclosed in a survey ¹¹ is to give transfusions to only those premature infants whose hemoglobin values have decreased to between 6 and 9 gm per 100 ml Our own practice has been to give transfusions to only those whose hemoglobin concentrations persist at levels of approximately 7 to 8 gm per 100 ml in the absence of significant reticulocytosis Transfusion is employed when there are evidences of infection anorexia listlessness and failure to gain weight We have found that premature infants rarely need transfusions on the basis of anemia alone When transfusions are required either packed erythrocytes or whole blood is given in amounts not to exceed 10 ml per kilogram of body weight

Limitations and Hazards of Transfusions Increased experience and investigation of untoward reactions have provided a greater measure of safety in the administration of transfusions Of the limitations and hazards included in the following list the most common are the hemolytic reactions serum hepatitis allergic and febrile reactions and circulatory overload

Development of irragroup 1 immunization

Hemolytic reactions

Incompatible blood

Old stored blood

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of transfusion to produce an anticipated rise in hemoglobin and erythrocytes in the absence of hemolysis or hemorrhage

Two recent sets of observations^{12, 13} document the thesis that transfusions administered at frequent intervals retard hemoglobin and erythrocyte formation. These data are based on an intensive study of a small group of children with severe Mediterranean anemia in whom transfusions could be interrupted following splenectomy. By the methods designed to permit quantitative differentiation between donor and recipient blood and by serial examinations of the total erythrocyte mass and circulating hemoglobin it was possible to demonstrate that a retarding effect occurred which was most marked from the first to the third week following transfusion. Not until donor blood had been entirely eliminated from the circulation were pretransfusion blood levels restored in the patient. The depressant effect of transfusion was further substantiated by another means. In patients with severe Mediterranean anemia the presence of large amounts of fetal hemoglobin provides a biologic tag of endogenous hemoglobin synthesis. Transfusions resulted in a depression of this component which increased sharply as donor cells left the circulation. The diminution in circulating fetal hemoglobin persisted in one patient for the first seven weeks after transfusion and in two others the decrease amounted to 50 per cent. Paralleling these observations is a recent report¹ that in patients with sickle cell anemia who received multiple transfusions a period of maximum depression of erythropoiesis occurred from the twelfth to the twenty fifth day with the percentage of sickle cells decreasing from 100 to 5 per cent. It is therefore advisable to withhold treatment occasionally in patients with chronic anemia to determine innate bone marrow function. In critical periods of growth such as in the anemia of prematurity it may be undesirable to interfere with bone marrow function by transfusion. In patients with erythroblastosis it has been suggested that a similar retardation can result from a persistent effort to maintain normal blood levels. These studies suggest the need for a less empirical and more individualized orientation not only in patients with refractory anemias but also in those with other disease conditions requiring frequent transfusions. The spacing and size of transfusions and blood levels to be attained require constant re-examination and appraisal.

Transfusions and Hemosiderosis Recent studies in iron metabolism have extended into an investigation of "iron overload" or hemosiderosis resulting from repeated transfusions. The total amount of iron in a normal adult approximates 4 to 5 gm. of which 15 to 20 per cent represents the iron reserve stored principally in the liver as ferritin and hemosiderin. In children storage iron normally represents 8.5 to 10.5 mg. per kilogram of body weight. In states of iron deficiency in infancy due to inadequate diet and periods of rapid growth storage depots are taxed and may be depleted. Iron otherwise accumulates and except for the normal excretion of approximately 1 mg. daily cannot leave the body except by bleeding. From the list on p. 75 it is apparent that excessive amounts of iron from destroyed erythrocytes become available for storage in patients receiving multiple transfusions (250 mg. of iron in 500 ml. of blood). Under such conditions hemosiderin deposits in normal storage depots increase greatly and in addition accessory sites assume the function of iron storage. Hemo-

Transmission of disease

Homologous serum jaundice (serum hepatitis)

Syphilis

Malaria

Circulatory overload

Excessive iron deposition

Hemosiderosis

Exogenous hemochromatosis

Suppression of hematopoiesis

Miscellaneous

Febrile reactions

Allergic reactions

Reactions from infected blood

Potassium toxicity

Cold agglutinins

Air embolism

Citric acid intoxication

Many advances have been made in the direction of reducing the icterogenic properties of plasma such as reduction of the size of the pool ultraviolet irradiation and the storage of liquid pooled plasma for prolonged periods at room temperature. The incidence of isosensitization from blood transfusion has been markedly reduced by preliminary screening and cross matching refinements of techniques and identification of increasing numbers of rare blood factors.

Circulatory Overload The administration of excessively large quantities of blood or even smaller amounts if given rapidly may precipitate cardiac failure from circulatory overload. In the course of a transfusion or shortly thereafter precordial pain, dyspnea, cyanosis and a dry cough are indicative of a rising venous pressure and pulmonary edema. In patients with severe anemia and evidences of congestive heart failure preliminary digitalization occasionally may be required. Packed or sedimented red cell suspensions are useful whenever it is essential to provide blood with the least possible disturbance to the patient's blood volume. Another expedient to prevent overloading the circulatory system is to give one half the circulated amount of blood in successive days.

In erythroblastosis death may result from cardiac failure caused by administering more blood than has been removed. Preliminary withdrawal of 30 to 50 ml of blood from the severely anemic infant may be necessary,¹⁴ or a deficit of even larger amounts (40 to 80 ml) should be established within a short time after beginning the exchange when venous pressures are excessively high.¹

Transfusions and Suppression of Blood Production One of the limitations of transfusion which deserves comment and which has as yet received scant attention is its potential depressant effects upon endogenous erythropoiesis and hemoglobin synthesis. That transfusions possess this secondary inhibitory effect was recognized in the treatment of pernicious anemia before the advent of specific therapy and has been confirmed more recently in several studies.^{4,6,10,11} Although retardation of hematopoiesis in minor degree may accompany a single transfusion it is overshadowed by the major corrective effects. In the case of multiple transfusions however this limiting influence may explain the failure

Transfusion Therapy in Hemorrhagic Disorders The intensive investigations of the hemorrhagic disorders have led to a clearer definition of the role of established and newly discovered factors in maintaining hemostasis. With the application of specialized procedures defects heretofore unknown have been described and appropriate treatment advised. These will be discussed under hemorrhagic diseases in Chapter 26. In general the patients with hemorrhagic disorders will benefit from fresh whole blood or plasma until the specific defect is determined.

Notes on Technique and Preservation of Blood When siliconized equipment is available its use is desirable for collection and administration of blood. A 20 gauge by 1 inch needle with a short sharp bevel is used in transfusion of patients in any age group.

In young children who require multiple transfusions such as those with leukemia or aplastic anemia suitable superficial veins often eventually become inaccessible or thrombosed. To avoid a cut down which may be accompanied by persistent oozing whole blood or plasma can be transfused into the proximal end of the tibia several inches below the tubercles. An appropriate bone marrow needle usually 18 gauge by 1 inch is inserted into the marrow as for aspiration and the distal plastic tip of the administration set is placed directly into the marrow needle. The flow is regulated as for intravenous administration with the bottle suspended at a level permitting flow by gravity. Because of the danger of infection it should be emphasized that this method of administering blood is used only as a last resort.

Acid citrate dextrose solution (ACD) is now generally used as an anticoagulant. The inclusion of dextrose in the preservation of blood slows the fall of diphosphoglyceric acid and adenosine triphosphate (ATP). The latter are involved in the energy producing mechanism of the red cell and permit its longer survival by maintaining red cell integrity.

During blood storage the leukocytes quickly disintegrate and lose their phagocytic power. The platelets disappear in about four days, antihemophilic globulin is progressively decreased¹² (in some instances as much as 10 per cent in the first week and approximately 50 per cent of the initial antihemophilic globulin after three weeks storage) and the prothrombin time is prolonged due to the loss of factor V. The use of plastic (Fenwall) bags retards the destruction of platelets. Platelet rich plasma obtained by this means has become increasingly popular in the treatment of serious bleeding in patients with thrombocytopenic purpura and leukemia.

In blood stored under refrigeration the potassium content of red cells decreases and that of the plasma increases both factors being intensified during prolonged storage. Marked electrocardiographic changes do not occur until the potassium level reaches approximately 8 mEq per liter.¹¹ Hyperkalemia induced in patients during exchange transfusions is transient but the potential risk of toxic effects should be kept in mind. The latter can be minimized by the use of relatively fresh blood, the removal of part of the plasma and the judicious use of calcium during replacement transfusions with citrated whole blood.¹³

siderosis refers to increased iron stores without tissue damage. Hemochromatosis is the development of tissue damage in persons with prolonged iron excess (see Chapter 12). The amount of iron present in organs such as the liver may exceed the amount given by repeated transfusions, indicating excessive absorption of iron from the gastrointestinal tract.³

Whether iron from transfused blood actually may produce hemochromatosis is still controversial, but there is evidence that excessive iron storage is potentially injurious.⁴ It must be understood that no unequivocal statement can be made as yet as to the extent or manner in which tissue siderosis is harmful. Studies in severe Cooley's anemia⁵ have shown, however, that hemosiderosis and fibrosis frequently coexist although there is no uniformity about the progression.

The development of true hepatic cirrhosis and fibrosis of the pancreas which characterizes hemochromatosis associated with transfusion hemosiderosis may depend upon the intervention of accessory factors such as continued hypoxia in addition to large iron deposits. Whether iron deposits alone are responsible for the hepatic damage in hemochromatosis is still debatable, but that it is a prerequisite for this pathologic process is more likely.

From these considerations it would seem that the iron derived from an occasional transfusion is harmless. A contrary situation may prevail, however, in the patient receiving multiple transfusions over long periods of time. In view of the risk involved, the most prudent course is to restrict the number of transfusions to the minimum compatible with comfort and moderate activity.

Allergic Reactions. It has been estimated that about 1 to 5 per cent of blood transfusions are followed by allergic reactions, principally urticaria and less often angioneurotic edema and asthma. These reactions are especially frequent in children with refractory anemias requiring multiple transfusions. In our

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In the last line of the text and in the second line of the title to Table 4 *exchange transfusions* should read *multiple transfusions*.

Table 4

Allergic Reactions to Exchange Transfusions

Aspirin	
Up to 5 years	1 grain (60 mg) per year
5 to 10 years	10 grains
Tripeleminamic (Pyribenzamine)	
Up to 5 years	25 mg
5 years and over	50 mg
Prednisone	
Regardless of age	10 mg given $\frac{1}{2}$ to 1 hour before transfusion

Other antihistamines may be substituted for Pyribenzamine

Jaundice—Differential Diagnosis in the Neonatal Period

The differential diagnosis of jaundice in the first weeks and months of life embraces a variety of conditions of a hemolytic and hepatic nature some of which are specific and others ill defined. These conditions may be interrelated to the extent that they appear as a hemolytic process at the outset but eventually give the clinical picture of liver disease. Common to most of these conditions in the first days of life is accelerated red cell destruction resulting in overproduction of bilirubin in amounts exceeding the excretory capacity of the liver for their disposal. On the other hand jaundice may date from the newborn period on a purely hepatic basis without significant intensity until the second or third week of life. Here malformations of the bile ducts or an inflammatory process accounts for the jaundice uncomplicated by a hemolytic component.

Erythroblastosis plays a prominent role in the differential diagnosis of jaundice both in the neonatal period when the disease is active and in the early months of life when it is an important factor in the pathogenesis of the inspissated bile syndrome. Since erythroblastosis will be dealt with fully in Chapters 9 and 10 in this discussion it will be referred to only in connection with syndromes from which it needs to be differentiated.

The pediatrician is confronted with making a diagnosis in infants with syndromes characterized by jaundice either during the first week of life (early neonatal period) as in those with erythroblastosis or in the remainder of the neonatal period (second to fourth weeks of life) as in those with prolonged obstructive jaundice.

Types of Bilirubin Different types of bilirubin have been identified with each process on the basis of their reaction with van den Bergh's diazo reagent. The serum from patients with bilirubinemia resulting from red cell destruction produces a predominantly delayed or indirect reaction whereas the serum from those with jaundice due to diseases of the liver and biliary system produces a direct (one minute) reaction. Bilirubinuria is absent in patients with hemolytic anemia in whom there is a retention of nonconjugated bilirubin (water insoluble form) in the plasma giving an indirect van den Bergh reaction. In patients with

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serum) generally between the first and second weeks of life. Immaturity of the liver previously held responsible for the failure to excrete bilirubin resulting from normal or slightly accelerated red cell destruction⁶ now finds more precise definition in the delayed development of an enzyme system required for the conversion of indirect to direct bilirubin.

Mechanical fragility of the red cells in the first days of life indicates their increased susceptibility to hemolysis.²² On the other hand two studies relating to the transfusion of placental blood into another infant, or an adult²³ indicate that fetal red cells have only a somewhat shorter survival than those of the adult. Only the red cells produced during late fetal life survive for a shorter time than expected.

The serum bilirubin of the full term infant seldom exceeds 10 mg per 100 ml of serum at its height on the second and third days of life and usually reaches no more than 7 mg per 100 ml. In the premature infant unaffected by erythroblastosis there is a tendency toward excessive bilirubinemia^{21, 24} as compared with the full term infant. The bilirubin level follows that of the full term infant in the first two days of life but then continues its rise to the fourth and sometimes fifth day before a decline occurs. The premature infant tends therefore to accumulate bilirubin beyond the two day period of the full term baby. Peak levels in the premature infant average 12 mg²¹ and may exceed 20 mg per 100 ml of serum.¹⁰ According to some authors hyperbilirubinemia exists in the premature infant if a level of more than 10 to 15 mg per 100 ml occurs during the first week of life. Physiologic jaundice is present in infants with lower levels.²⁷

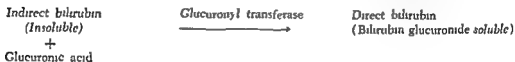
Jaundice in the first twenty four hours of life is never physiologic. In infants with erythroblastosis in contrast to those with physiologic icterus jaundice develops rapidly with high bilirubin levels on the first day of life and maximum concentrations often reached in less than two days. In the absence of isoimmunization excessive bilirubin levels in the full term or premature infant develop more slowly and usually present initial problems of management beyond the first forty eight hours of life.

Hyperbilirubinemia of the Newborn Infant Unrelated to Isoimmunization
Hyperbilirubinemia unrelated to erythroblastosis may reach a magnitude in the full term and the premature infant sufficient to place the infant in jeopardy of brain damage. In a series of such infants bilirubin levels (indirect reacting) ranged from 12 mg per 100 ml on the first day of life to 20 mg and above (maximum 32 mg) during the ensuing week.¹¹ The peak level was reached earlier in the week in the full term infant than in the premature infant. The degree of hyperbilirubinemia is regarded as an accentuation of the mechanism involved in the pathogenesis of physiologic jaundice. The premature infant particularly requires careful watching because of the tendency toward excessive bilirubinemia and subsequent kernicterus.

In this group of patients with nonerythroblastic hyperbilirubinemia there are conflicting views as to the critical levels for exchange transfusion or even for its routine use. The low incidence of kernicterus in untreated premature infants (less than 1 per cent) led one group of investigators to conclude that preventive treatment of kernicterus should be on an individual rather than a routine basis.²⁸ This was based on the fact that the existence of a critical peak concentration of

obstructive and hepatogenous jaundice in whom the plasma contains largely conjugated bilirubin (water soluble form) giving a predominantly direct van den Bergh reaction bilirubinuria is present

Enzymatic Conversion of Indirect to Direct Bilirubin Indirect bilirubin formed by the reticuloendothelial cells is transported in the plasma attached to proteins mainly albumin. There is evidence^{7,8} that the excretion of water insoluble free indirect bilirubin depends upon its conversion to a direct reacting water soluble pigment and that the main pathway is through conjugation with glucuronic acid. It has also been demonstrated that the formation of bilirubin glucuronide in liver depends upon the transfer by an enzyme in liver microsomes (transferase) of glucuronic acid from uridine diphosphate glucuronic acid. Free glucuronic acid however does not enter into the conjugation of indirect bilirubin. This conversion may be schematically represented as follows



According to this concept before birth the placenta and not the liver is responsible for removal of bilirubin from the fetal blood.⁴ The infant therefore is not born jaundiced and only becomes so if the enzyme system responsible for the conjugation of bilirubin has not yet been fully developed. It may be assumed that the fall in pigment concentration during the first week of life depends upon a corresponding rise in bilirubin transferase activity toward adult levels.⁹

The effect of this enzyme deficiency is manifested particularly in patients with erythroblastosis as a result of which the newborn infant is exposed to high plasma concentrations of bilirubin. Excessive hyperbilirubinemia occurring occasionally in the full term and premature infant in connection with physiologic jaundice and unrelated to blood group incompatibility is based on the same difficulty of converting indirect to direct bilirubin.¹⁰ An unidentified substance in the plasma of the pregnant woman which inhibits the *in vitro* conversion of indirect bilirubin to direct bilirubin has been demonstrated by rat liver slices. This inhibitor may conceivably contribute to the development of hyperbilirubinemia in the newborn infant.^{36a}

Conditions such as Gilbert's disease,⁹ familial nonhemolytic jaundice¹⁴ and the Crigler-Najjar syndrome¹ in which the jaundice is due to the accumulation of indirect (nonconjugated) bilirubin may now be explained as a failure of the liver to form direct bilirubin (bilirubin glucuronide) because of a deficiency of the specific enzyme transferase.

JAUNDICE IN THE EARLY NEONATAL PERIOD

Physiologic Jaundice (Icterus Neonatorum) Jaundice appears commonly in newborn infants from the second to the fifth day of life. After reaching a maximum intensity between the second and third days the jaundice begins to diminish with bilirubin dropping to normal levels (0.25 to 0.75 mg per 100 ml of

Hereditary Nonspherocytic Hemolytic Disease Hereditary nonspherocytic hemolytic disease may also present itself at birth with the clinical picture of erythroblastosis.¹¹ Absence of spherocytosis, normal osmotic fragility, and failure to respond to splenectomy rule out hereditary spherocytosis. A negative Coombs test and the absence of Rh incompatibility eliminate erythroblastosis, and persistence of the jaundice rules out ABO involvement.

Elliptocytic (Ovalocytic) Hemolytic Anemia Elliptocytic (ovalocytic) hemolytic anemia associated with jaundice on the second day of life has been reported but anemia was not noted until the infant was 1 month of age.³⁷

Heinz Body Anemia in the Newborn Infant Heinz body anemia in the newborn infant has been described as a hemolytic anemia occurring in premature infants.⁹ These babies develop a nonobstructive jaundice in the first few days of life, usually between the first and fifth days. Anemia appears in the second to the third week as the jaundice fades. The Coombs test is negative. Examination of the blood before the development of the anemia shows a progressive increase in the number of Heinz bodies (9 to 45 per cent of the red cells). Originally described in premature and congenitally defective infants, Heinz body anemia has also been reported in full term infants.⁴¹

Heinz bodies are refractile intracellular inclusions in the red cells which are readily seen in fresh wet preparations or in preparations with supravital dyes such as brilliant cresyl blue. They are composed of denatured globin and serve as an indication of a hemolytic process. The blood smear shows anisocytosis, target cells, irregularly shaped fragmenting cells, microspherocytes, and basophilic stippling.

The disease is self limited, provided the child survives the original hemolytic episode. No evidence of exogenous toxic substances are found before, during, or after birth. High dosages of vitamin K (Synkavite) have been incriminated as a possible cause of Heinz body anemia, since severe hemolytic anemia with some Heinz body formation can be produced experimentally in animals by administration of relatively large doses of Synkavite.⁴ Another possibility is the existence of an inherent defect in red cells rendering them susceptible to hemolysis analogous to primaquine sensitivity, in which Heinz bodies also occur.

Acute Hemolytic Anemia Related to Naphthalene The ingestion of naphthalene (moth balls and flakes) has been described in children as a cause of severe illness with fever, nausea, diarrhea, and evidences of an acute hemolytic anemia. The initial cases were described in four Negro infants.⁶ Fatal naphthalene intoxication resulting from absorption through the skin from diapers stored in moth balls and crystals has been reported in newborn infants.⁴¹ Profound anemia, hemoglobinuria, hemoglobinemia, normoblastemia, methemoglobinemia, leukocytosis, jaundice, cyanosis, spherocytosis, occasional Heinz bodies, and hemoglobinuria are noted. Whole blood or packed cells are essential in treatment.¹⁵

As is true of primaquine sensitivity, the susceptibility to naphthalene poisoning depends upon an intrinsic and genetically determined defect of the red cells which renders them especially susceptible to hemolysis (see Chapter 15). The abnormality is in glutathione metabolism which expresses itself in the older erythrocytes. As stated elsewhere, the basic biochemical defect is a deficiency

plasma bilirubin in premature infants above which kernicterus could be expected to develop could not be demonstrated in their series. Nevertheless levels of 20 mg and more of indirect bilirubin serve as well established guide posts for orientation and management in this group as in that with erythroblastosis. The treatment of this type of hyperbilirubinemia will be discussed further in Chapter 10.

Relation of Vitamin K to Hyperbilirubinemia, Kernicterus, and Hemolytic Anemia. Large intramuscular doses of a vitamin K preparation (Synkavite) have been shown to increase the serum bilirubin levels in infants during the first week of life.⁴³ The most severe bilirubinemia hence the risk of kernicterus tends to occur in the premature infant in whom elevated bilirubin levels are normally prone to exist.⁴ Large dosages of vitamin K are also hepatotoxic, hemolytic or both. The liberation of excessive amounts of bilirubin resulting from increased hemolysis in the newborn premature infant following excessive vitamin K dosage has been demonstrated.⁴ Because of these possibilities it is unwise to exceed stated dosages for the prophylaxis and treatment of hemorrhagic disease of the newborn infant. There is ample evidence that a single dose of a water soluble analogue equivalent to 1 mg of synthetic vitamin K (menadione) is adequate to prevent hemorrhagic disease in the newborn infant. This would correspond to 3 mg of menadione sodium diphosphate (Synkavite) vitamin K analogue.¹

It has been observed⁴⁴ that the parenteral administration of a large amount of a vitamin K analogue (Hykinone) to mothers during labor resulted in marked early hyperbilirubinemia in newborn premature infants which caused central nervous system involvement in several cases.

The hemolytic effects of vitamin K have been explained on the basis of an inherent glutathione instability exhibited by the red cells of newborn infants. Incubation of the red cells of newborn infants with vitamin K analogues results in a characteristic fall in glutathione content which accounts for their *in vivo* hemolysis.⁴⁵ This phenomenon corresponds with the susceptibility to hemolysis of selected persons who are sensitive to primaquine and naphthalene.

Hereditary Spherocytosis. Hereditary spherocytosis is manifested occasionally in the newborn period⁴⁶⁻⁴⁸ at which time it may be confused with erythroblastosis. Microspherocytosis is present in patients with both hereditary spherocytosis and ABO (not Rh) erythroblastosis. In those with erythroblastosis the microspherocytosis lasts a few days whereas it persists in those with hereditary spherocytosis. Incompatibility between the blood groups of the mother and fetus and frequently a positive direct and indirect Coombs test characterize erythroblastosis. In patients with hereditary spherocytosis the Coombs test is uniformly negative and a positive family history is common and an increased erythrocyte fragility is noted in the blood of affected persons.

Exchange transfusions to prevent kernicterus are indicated for the treatment of patients with hyperbilirubinemia as in those with erythroblastosis. It is as important to remove congenitally defective and readily destructible red cells from the blood of patients with spherocytic anemia as it is to remove sensitized cells from the blood of those with erythroblastosis.

pain fatigue dark urine and slight enlargement and tenderness of the liver. The direct bilirubin accounts for about 60 per cent of the total serum bilirubin. The liver cell is apparently able to conjugate indirect bilirubin with glucuronic acid and convert it to direct bilirubin but cannot excrete it into the bile. The most striking finding in all cases is the presence of a coarsely granular brown pigment in a sharp centrilobular distribution in the parenchymal cells. A familial history of jaundice has been occasionally obtained in siblings.¹⁵ This type of bilirubinemia in which the direct bilirubin fraction predominates is to be differentiated from Gilbert's disease,¹⁶ a familial nonhemolytic jaundice of Dimeshek and Singer,¹⁸ and the Crigler-Najjar syndrome¹⁷ in which the indirect reacting bilirubin is in excess. Although the Dubin-Johnson syndrome has been observed principally in adults it has been recorded occasionally in infancy but not specifically in the early or late neonatal period.

We have recently encountered an infant jaundiced from birth with blood groups compatible with those of the mother who probably had a variant of this disease. The Coombs test was negative. In the first two weeks of life the total bilirubin ranged from 47 to 59 mg per cent with a direct fraction of 33 to 40 mg and an indirect fraction of 13 to 19 mg. Although the Coombs test was negative the infant was given an exchange transfusion on the third day of life because of the mounting indirect fraction. The mother, her sister and her two brothers have been chronically jaundiced but in good health. Their total bilirubin ranged from 11 to 13 mg per cent of which the direct reacting fraction ranged from 4 to 11 mg and the indirect from 1.3 to 4.1 mg per cent. Liver biopsy in the mother however showed no pathognomonic pigmentation of the parenchymal cells as described in the Dubin-Johnson syndrome. The infant's jaundice and bilirubinemia receded over the period of several months.

A closely allied disease but without pigment changes in the liver is that described by Rotor.²⁰ This too is a familial type of nonhemolytic jaundice characterized by a direct van den Bergh reaction. The case described may fall into this category.

JAUNDICE IN THE LATER NEONATAL PERIOD

Prolonged Obstructive Jaundice. Several diseases of early infancy are characterized by evidence of biliary obstruction which finds its inception in the first weeks of life. Patients with diseases associated with biliary obstruction and those with absence of the bile ducts have been designated as members of a group with prolonged obstructive jaundice. Individual diagnosis is usually difficult but complexities are resolved at times by scrupulous and continuous appraisal of clinical and laboratory findings.

This group of cases has been classified by Hsia and co-workers²¹ into the following categories: biliary obstruction caused by inspissation of bile either from hemolysis as occurs in erythroblastosis or of multiple etiologies and congenital atresia of the intrahepatic and extrahepatic ducts including the common bile duct. Neonatal hepatitis has been included as one of the causative factors in the inspissated bile syndrome of nonerythroblastic origin.

Prolonged obstructive jaundice is characterized by jaundice increasing after the first week of life but waning in its intensity after a maximum has been reached. Additional features are light stools, dark urine, elevation of both total and direct serum bilirubin levels, decreased urobilinogen in the urine and stool and moderate to marked enlargement of the liver and spleen. Whereas the level

of glucose 6 phosphate dehydrogenase. This enzyme is essential in maintaining glutathione in a reduced state which is necessary to protect intracellular hemoglobin from the toxic effects of a number of drugs. These defects were described in two newborn infants with hemolytic anemia who had been exposed to articles of clothing impregnated with moth balls.¹² In another case¹³ naphthalene and its metabolites passed through the placenta to the newborn infant producing jaundice and evidences of a hemolytic anemia. The mother who had ingested moth balls had a profound hemolytic anemia. An exchange transfusion in the infant was performed when the indirect bilirubin rose to nearly 20 mg per 100 ml from which point the course was uneventful.

Infections in Newborn Infants Icterus associated with congenital syphilis, sepsis,¹⁴ toxoplasmosis and cytomegalic inclusion disease may result from excessive blood destruction as well as from liver cell damage. Pneumonitis, congenital heart disease, meningitis, urinary tract infection and intracranial hemorrhage are some of the pathologic states associated with hyperbilirubinemia. The elevation of both the direct and indirect serum bilirubin levels in patients with these conditions reflects excessive hemolysis of red cells as well as hepatocellular damage.¹⁵

Cytomegalic Inclusion Disease Cytomegalic inclusion disease which may appear at or shortly after birth should be suspected especially in the prematurely born infant in the presence of jaundice, purpura, hepatosplenomegaly and neurologic disturbances.¹⁶ Other features include hemolytic anemia, thrombocytopenia, petechiae, hematuria, erythroblastemia, pneumonitis and cerebral calcification.

The disease is characterized pathologically by an inclusion containing giant cell appearing predominantly in the lining of the ducts of various organs such as the alveoli of the lungs, tubular epithelium of the kidney and the brain. The diagnostic cytomegalic or owl-eyed cells with the large intracytoplasmic or intranuclear inclusion bodies are found in the sediment of urine kept under refrigeration¹⁷ and in gastric washings.¹⁸ The infectious agent has been ascribed to a species specific salivary gland virus. Cytopathogenicity of this virus obtained from the mouth and urine for tissue cultures of human fibroblasts provides a means for study of the human infection with this virus.¹⁹

Congenital Toxoplasmosis Congenital toxoplasmosis is a rare cause of neonatal jaundice but is probably more frequent than is suspected. This disease may be confused at the onset with erythroblastosis fetalis.²⁰ Congenital toxoplasmosis may be associated with deep jaundice and in a recently observed infant the serum bilirubin totaled 16 mg per 100 ml of which 13 mg was of the indirect type. A normal hemoglobin level, a negative Coombs test and absence of intragroup incompatibility help to differentiate this condition from erythroblastosis. In the active disease the parasites have been demonstrated in smears of the spinal fluid. The diagnosis can also be confirmed by serologic tests of various types.²¹

Chronic Idiopathic Jaundice (Dubin Johnson Type, Dubin Rejnitz Disease) Chronic idiopathic jaundice of Dubin Johnson type^{22,23} manifests itself as a form of chronic or intermittent jaundice of fluctuating intensity with abdominal

pain fatigue dark urine and slight enlargement and tenderness of the liver. The direct bilirubin accounts for about 60 per cent of the total serum bilirubin. The liver cell is apparently able to conjugate indirect bilirubin with glucuronic acid and convert it to direct bilirubin but cannot excrete it into the bile. The most striking finding in all cases is the presence of a coarsely granular brown pigment in a sharp centrilobular distribution in the parenchymal cells. A familial history of jaundice has been occasionally obtained in siblings.³ This type of bilirubinemia in which the direct bilirubin fraction predominates is to be differentiated from Gilbert's disease,¹³ familial nonhemolytic jaundice of Dameshek and Singer¹⁸ and the Crigler-Najjar syndrome¹⁷ in which the indirect reacting bilirubin is in excess. Although the Dubin-Johnson syndrome has been observed principally in adults it has been recorded occasionally in infancy but not specifically in the early or late neonatal period.

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of serum bilirubin remains relatively constant in infants with biliary atresia it may fluctuate in those with the other groups of disease. Extensive erythropoiesis is found in the liver of patients with the inspissated bile syndrome due to erythroblastosis but none is found in patients with the other syndromes. The liver flocculation tests for liver function are negative but may be transiently positive. No single laboratory test can be used for specific differentiation. Individualization is necessary and the course in each patient must be followed clinically and with a combination of diagnostic laboratory tests.

Obstructive Jaundice Complicating Erythroblastosis—Inspissated Bile Syndrome The jaundice in most infants with erythroblastosis fetalis begins to subside by the fourth or fifth day and terminates by the end of ten days. Occasionally the jaundice persists, the stools become pale and bile appears in the urine, the entire picture closely simulating congenital biliary atresia. The obstructive phenomenon may be of short or long duration. When prolonged it acquires the clinical and pathologic characteristics of the inspissated bile syndrome although qualitatively the basic features are similar regardless of duration.

Obstructive jaundice may occur at the height of the hemolytic process in erythroblastosis or not until some weeks later. Occasionally an evanescent phase of hemolytic jaundice occurs soon after birth with a recurrence of the jaundice associated with biliary obstruction after a few weeks.⁴ When obstruction appears in the first days of life the total serum bilirubin becomes progressively elevated mainly due to the direct fraction and then recedes slowly to normal levels within a few weeks. When it persists at intermediate levels for prolonged periods it is categorized as obstructive jaundice due to the inspissated bile syndrome.

Exchange transfusions have a negligible effect on the percentage of direct bilirubin and do not alter the progression of biliary obstruction.²³ This procedure should be carried out, however, if there is a significant elevation of indirect reacting pigment which in contrast to direct reacting bilirubin carries the hazard of cerebral damage. In the reported cases¹⁹ the elevated direct serum bilirubin level decreased in most patients spontaneously from eighteen hours to six days. It is of interest that the levels of direct bilirubin in the cord blood were higher than those in normal blood (approximately 1 mg per 100 ml).

In a newborn infant under our observation the cord blood bilirubin rose from a total of 17 to 48 mg with 12 to 35 mg direct reacting and 5 to 13 mg indirect reacting pigment by the fourth day of life. The next day this value is lower and continued to decrease during the following week although normal blood levels were not achieved until the second month. In another infant who was not seen until the fifth day of life liver involvement was apparent when the total bilirubin on that day measured 43 mg of which 31 mg was direct and 12 mg indirect. On the seventh day the total bilirubin was 121 mg of which 83 mg was the direct fraction and 38 mg indirect. Jaundice then began to recede and by the third week the total bilirubin measured 17 mg per cent the direct fraction 14 mg and indirect 3 mg. In the ninth week there was a total of 7 mg with 6 mg indirect. Except for a slight transient elevation of thymol turbidity in the second month there was no evidence of liver involvement. At 13 months of age the infant was still normal without signs of nervous system involvement.

The degree of biliary obstruction is usually partial—rarely complete. Jaundice occurs between 3 weeks and 6 months of age with an average duration of seven to eight weeks.³¹ In cases of prolonged jaundice extending into the second month there is a progressive increase of direct as well as indirect bilirubin. The precise reason for the inspissated bile syndrome complicating erythroblastosis is unknown whether it be due to hepatocellular damage incurred by the hemolytic disease or to the overload of pigment causing stasis and complete block. Multinucleated giant liver cells found in neonatal hepatitis have also been described in this condition. Recovery has been ascribed to cessation of the hemolytic process and enlargement of biliary ducts with increasing age.³¹

In differentiating the inspissated bile syndrome from other categories of obstructive jaundice the history, clinical observation and laboratory tests are helpful. Patients with the inspissated bile syndrome due to erythroblastosis demonstrate the following clinical and laboratory features: clinical evidence of erythroblastosis during the early acute phase; jaundice on the first day of life which persists longer than the first three weeks; an obstructive type of jaundice with marked elevation in both direct and indirect bilirubin levels; negative liver flocculation tests; and demonstration of maternal fetal blood group incompatibilities.³⁴ Spontaneous recovery is the rule in patients with obstructive jaundice in this category.

Inspissated Bile Syndrome of Unknown Etiology No single factor has been established for the etiology of the nonerythroblastotic type of the inspissated bile syndrome and the exact limits of this classification have not been defined. Neonatal hepatitis has long been regarded as the basic pathology but no unequivocal evidence has been offered for its causation by the agents of homologous serum hepatitis or the virus of infectious hepatitis.³ Focal or widespread inflammatory degeneration is found in the liver with formation of multinucleated giant hepatic cells.³⁵ This morphologic change has been regarded however as the major abnormality rather than as a nonspecific finding in this syndrome. Plugging by inspissated bile in biliary canaliculi is of secondary importance.⁹ This giant cell transformation has also been observed however with intrahepatic and extrahepatic abnormalities and has led to the hypothesis that neonatal hepatitis represents a development defect of liver cells resulting in aplasia of the bile capillaries and malformation of liver cells.⁷ The relation of these morphologic changes to neonatal hepatitis is by no means settled. Factors such as small bile ducts, dehydration and hepatic immaturity contribute to biliary stasis. The recovery of an inclusion cell virus from three infants with neonatal hepatitis³³ suggests such an etiology for a group of cases of this disorder.

The urine contains bile and the stools are acholic, green or yellow. The flocculation tests of liver function are usually negative. The direct and total serum bilirubin levels are always elevated and show either a slowly falling or variable trend.

Patients in this category are often noted to be poorly nourished as compared with those in other categories who exhibit good health. About 60 per cent of these infants recover without sequelae; some develop cirrhosis and other die.

of surgical complications.³ A recessive mode of inheritance has been suggested in this syndrome.¹¹

Atresia of the Bile Ducts Congenital atresia of the extrahepatic bile ducts represents about 60 per cent of cases of prolonged obstructive jaundice in infancy.⁷ Despite persistent and progressive jaundice, affected infants appear well nourished in the first three or four months of life. The stools are acholic, the urine is dark, and the level of bilirubin is constant. It has been estimated that of about 20 per cent of patients amenable to surgical anastomosis, only 2 to 3 per cent of the total with chronic obstructive jaundice due to atresia of the extrahepatic duct system have been treated successfully.¹² The remainder succumb in the immediate postoperative period or later to biliary cirrhosis.

Congenital atresia of intrahepatic ducts may occur with or without an associated extrahepatic atresia. Diagnosis is only possible by biopsy. In general the findings resemble those in patients with congenital atresia of the large extrahepatic bile ducts. In a group of patients in whom atresia of both intrahepatic and extrahepatic ducts occurred together the course was more benign with a life span of three to five years.¹³ It was later demonstrated that the intrahepatic bile ducts are present in the early months of life and disappear coincident with prolonged disturbance in bilirubin elimination through the hepatic cells into the biliary canaliculi, giving histologic evidence of cholestasis.¹⁴

Management of Prolonged Obstructive Jaundice Although evaluation from clinical and laboratory sources may be helpful, exploratory laparotomy is sometimes undertaken when jaundice is unduly prolonged. The problem becomes increasingly perplexing from the second to the fourth months of life when the type of liver involvement is not discernible and the possibility, even though vague, of biliary atresia is entertained. One of the drawbacks to early surgical intervention is the finding that such a procedure in infants under 3 months of age with liver damage is attended with serious risk.¹⁵

Gellis and Hsia¹⁶ have provided indications for surgical exploration that are eminently practical. Operation is indicated if there is a consistent absence of bile in the stools and duodenal secretions and of urobilinogen in the urine; if the initial bilirubin level is low and shows a slowly progressive increase with no variability in pattern; if the flocculation tests are negative; and if there is no blood group incompatibility to suggest erythroblastosis. Operation should be deferred longer if there is bile in the stools or duodenal secretions, even in small amounts; if urobilinogen is present in the urine in normal dilutions; if the initial bilirubin level is high and has shown a tendency to fall or be highly variable with no definite upward trend; if the flocculation tests are positive; and if blood group incompatibility suggesting erythroblastosis can be demonstrated.

By examining liver tissue in cases of biliary atresia, Christy and Boley¹⁷ have found that a large amount of fibrosis was already present within the first two months after birth and that it tended to increase with age. Instead of extensive abdominal explorations which are hazardous, Swenson and Fisher¹⁸ have described a limited exploratory operation with direct cholangiography utilizing the gall bladder and outlining the extrahepatic duct system with Diodrast. A liver biopsy is made at the same time. Subsequent extensive exploration

is undertaken if no hepatic or common duct is visualized. With this program an exploratory procedure and correction can be undertaken before the third month of life.

Medical treatment with magnesium sulfate and other choleragogues to establish an increased flow of bile has been reported¹⁴ but has thus far proved unsuccessful. Steroids given orally occasionally will lower the bilirubin level in patients without biliary atresia but experience with their use is still limited.

Galactosemia Galactosemia almost always is accompanied by obstructive jaundice during the first week of life and persists for a variable length of time thereafter. In the absence of anemia, hepatomegaly, and a positive reaction for sugar warrant an examination for galactosemia. (The tape test does not detect galactose in the urine.) The congenital inability to convert galactose to glycogen in the liver is corrected by feeding the infant a milk substitute.^{15,16}

Congenital Familial Nonhemolytic Jaundice With Kernicterus Congenital familial nonhemolytic jaundice with kernicterus has been reported by Crigler and Najjar.¹⁷ Six of eight infants in a single large family had jaundice with severe neurologic disease from the second day of life until death, which usually occurred within the first year of life. There was no incompatibility of blood groups or evidence of hemolytic disease. The indirect serum bilirubin level was constantly elevated, with minimal elevations in the direct fraction. The affected infants showed evidence of kernicterus between 2 weeks and 3 months of age.

The abnormality depends upon a genetic defect consisting of an inability of hepatic cells to metabolize and excrete bilirubin. There is evidence to show that the abnormally high levels of indirect bilirubin represent defective conjugation with glucuronic acid required for conversion to soluble direct bilirubin. These patients presumably are deficient in the same bilirubin glucuronyl transferase enzyme that is slow to develop in the newborn infant.

Exceptions to the occurrence of kernicterus were seen in two of the children in this family, now 2 and 5 years of age, who have had persistent jaundice from birth but who have shown no neurologic disability.¹⁸ In another case of this disease, not in the same family, neurologic symptoms did not appear until the patient was 3 years of age¹⁹ despite the persistence of jaundice from birth.

Except for the development of kernicterus, this condition corresponds to a type of retention jaundice which may be readily confused with chronic hemolytic jaundice—namely, *familial nonhemolytic jaundice*.¹⁸ It represents a form of constitutional hepatocellular dysfunction resulting in an accumulation of indirect bilirubin.

Anemia, microcytosis, spherocytosis, reticulocytosis, increased fragility of the red cells, splenomegaly, and increased excretion of urobilinogen in the urine and stools, which characterize congenital spherocytic anemia, are absent in this condition. Jaundice in this form of familial nonhemolytic jaundice usually appears soon after birth and persists throughout life. The concentration of bilirubin in the serum seldom exceeds 5 mg per 100 ml, with a range of 12 to 15 mg per cent, and is practically all of the indirect type. In most instances the disease is found in young adults and follows a benign clinical course without bilirubin encephalopathy. The Crigler-Najjar syndrome¹⁷ on the other hand is associated

with severe hyperbilirubinemia with 10 to 44 mg of serum bilirubin per 100 ml with the greater portion primarily of the indirect type.

Jaundice and Carotenemia Carotenemia occasionally confused with jaundice refers to a harmless yellowish discoloration of the skin which occurs in infants and young children due to increased hypochromes in the blood from ingestion of carrots squash spinach egg yolk and other sources of dietary pigment. The discoloration appears especially in the skin of the palms soles forehead and nasolabial folds. In contrast to jaundice it is absent from the conjunctivae and buccal mucous membranes. The icterus index is elevated but the van den Bergh reaction is negative.¹

Jaundice Due to Pyloric Stenosis Jaundice has been described²¹ with a marked increase in the indirect reacting bilirubin in the serum of patients with pyloric stenosis due to posterior angulation of the pyloric tumor and extrinsic pressure on the common bile duct. With operative treatment jaundice disappeared promptly. This combination assumes importance only because of the need for differentiation from other contemporaneous types of obstructive jaundice.

Jaundice and Hypothyroidism Prolonged physiologic jaundice has been encountered in connection with congenital cretinism. This association may lead to an early diagnosis and treatment of the hypothyroid condition which might otherwise not have been suspected.

Hematomas Mild jaundice occasionally follows absorption of bilirubin from hematomas in diverse locations including those in intracranial and subdural sites.

Miscellaneous In addition to cretinism and pyloric stenosis prolonged physiologic jaundice may be due to other abnormalities such as congenital heart lesions and kidney disease.²²

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subsequent pregnancy with an Rh positive fetus. This means that a healthy child usually results from the first pregnancy and that subsequent children are often affected since later pregnancies provide an effective stimulus to an antibody-forming mechanism set in motion by the first pregnancy.

Clinical Features The diverse symptomatology of erythroblastosis fetalis reflects variations in the degree of red cell destruction, blood production, and extramedullary hemopoiesis in the liver function. In about 15 to 20 per cent of Rh positive infants of Rh negative immunized mothers, clinical illness will be absent or hardly perceptible. The intensity of the hemolytic process is influenced largely by the titer of maternal anti Rh antibodies. A high titer (usually 1:64 and over) or a rising titer carries a serious import and is reflected in the severity of the anemia and depth of jaundice.

The clinical findings include jaundice, anemia, enlargement of the liver and spleen, petechiae, and in severe cases hemorrhages and edema. The liver and spleen may be moderately enlarged at birth or may become palpable as the disease progresses in the first week of life. The disease is classified according to increasing severity: congenital anemia of the newborn infant, the mild form in which pallor becomes apparent as jaundice fades in the first week of life; icterus gravis neonatorum, in which the infant is more seriously affected with progressive anemia and jaundice; and hydrops fetalis (universal edema, generalized edema of the newborn infant), the stage in which the stillborn infant is often markedly edematous. Except for hydrops fetalis, the term erythroblastosis, qualified by the degree of severity, has supplanted the terminology of the first two categories. They represent each end of a continuous range of increasing severity with marked variation in the two salient features of the disease—namely, anemia and jaundice.

The hydropic infant, although usually stillborn and premature, may be born alive but seldom survives more than a few hours. The massive edema is attributed to heart failure and pulmonary edema. Placental excretion of bile pigments in utero supposedly accounts for the lack of jaundice in these infants. Mild edema is not necessarily fatal since survival is possible with active treatment.

In the infant with erythroblastosis, jaundice of the skin and sclerae is almost never present at birth but appears in the first hours of life. Although the severity of the hemolytic process cannot be predicted accurately by examination of the amniotic fluid, the latter is often pale yellow or yellowish brown and the vernix normal or dark yellow. In the latter case, the skin underlying the discolored vernix is usually not icteric. Edema occurs as a complication in the severely anemic infant with congestive heart failure.

Detection of Early Jaundice of the Newborn Infant The need for the early detection of jaundice has been emphasized in infants with erythroblastosis not caused by the Rh factor, in whom jaundice is not anticipated, such as in those with immunization by A₁ and rare blood factors. In the newborn infant, jaundice is generally not apparent until the blood level reaches 4 to 6 mg. per 100 ml. The absence of jaundice at lower levels may be due to differences in capillary permeability in the newborn baby as compared with the adult or may be due

Erythroblastosis Fetalis (Hemolytic Anemia of the Newborn Infant)—General Considerations

Definition Erythroblastosis fetalis is a disease of the fetus and newborn infant characterized by a hemolytic anemia based on incompatibility between the blood group of the mother and that of her offspring. The disease is designated as such because of the frequent appearance of nucleated red cells in the peripheral blood resulting from their active proliferation in the liver, spleen and bone marrow as a compensation for excessive hemolysis.

Pathogenesis Isoimmunization results from the presence of an antigenic substance in the fetal red blood cell that is not present in the mother's red blood cells. Entrance of this antigenic factor into the maternal circulation either by direct transfusion or by transfer across the placenta from the fetal circulation results in the elaboration of a specific antibody by the mother. The transplacental transfer of red cells or antigenic substance is facilitated by the existence in man of a single syncytial cell layer separating the villi containing fetal blood vessels and the maternal sinuses by the thinning out of the expanding placental surfaces and by small leaks in the placental barrier which permit leakage of fetal blood.

Sensitization during pregnancies occurs classically when fetal Rh positive blood enters the circulation of the Rh negative mother. A similar mechanism is postulated with respect to the ABO groups in heterospecific pregnancies. Maternal antibodies recross the placenta gaining access to the fetal circulation where they become attached to fetal red cells resulting in their removal from the circulation and subsequent destruction. The various pathologic clinical and hematologic manifestations of erythroblastosis stem from the reaction of these antibodies with fetal red cells in utero and in the neonatal period. It has been estimated that only minute amounts of fetal blood (0.03 to 0.07 ml.) are required for immunization.³

A mother sensitized by previous transfusions is likely to have an infant with erythroblastosis in her first pregnancy, whereas the first born of a mother immunized during pregnancy is usually free of the disease. In the latter situation anti Rh antibodies cannot be detected in the maternal circulation until there is a

subsequent pregnancy with an Rh positive fetus. This means that a healthy child usually results from the first pregnancy and that subsequent children are often affected since later pregnancies provide an effective stimulus to an antibody-forming mechanism set in motion by the first pregnancy.

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to a diminished affinity to pigment in the skin of newborn infants.¹⁹ To safeguard those in whom jaundice is not anticipated it is suggested that all infants should be inspected frequently during the first two days of life. As aids for the detection of jaundice Allen²⁰ advises artificial lighting of a specific type and the use of a polished piece of Lucite for blanching the skin.

Kernicterus Brain damage (kernicterus) represents the most serious complication of erythroblastosis and is associated with high concentrations of indirect bilirubin from any cause occurring in the first two weeks of life. The extent of potential bilirubin accumulation which may be involved in erythroblastosis can be estimated. Each gram of hemoglobin constitutes a potential source of 35 mg of bilirubin. For an infant weighing 3 kg, a decrease of 1 gm of hemoglobin per 100 ml of blood represents the destruction of 3 gm of hemoglobin and the source of 105 mg of bilirubin. A newborn infant handicapped by immature liver enzyme systems therefore may accumulate toxic amounts of bilirubin without evidence of marked anemia.

Kernicterus thus can be regarded as a bilirubin encephalopathy. Cerebral involvement should be suspected in an infant thirty six hours or more after birth who feeds poorly, becomes lethargic and develops muscular twitching or whose cry is sharp and high pitched. Alteration of the Moro reflex displayed in response to jarring of the crib or a loud noise from the normal embrace reflex of the arms with fists clenched in the affected infant is significant.³ Opisthotonus and respiratory failure follow in the severe and fatal cases. Signs of nervous system involvement should be sought in severely affected full term infants in whom jaundice reaches a peak intensity at thirty six hours and in severely affected premature infants in whom jaundice reaches a peak intensity by the fifth or sixth day.

Kernicterus has been established as a postnatal complication and is almost completely preventable. Although there is a variation in susceptibility the likelihood of kernicterus in the infant with erythroblastosis when indirect bilirubin concentrations reach 20 mg per cent and above has been well documented. Signs of neurologic damage appear thirty six hours or more after birth.

Assiduous attention to maintaining serum bilirubin concentrations at levels below 20 mg per cent by one or more exchange transfusions has led to a remarkable reduction in the incidence of kernicterus. Since kernicterus is correlated with the intensity of bilirubinemia it is important to observe closely those infants who are severely affected clinically at birth and whose cord blood shows elevated bilirubin levels and lowered hemoglobin content. This is especially important in immature infants and in male infants with a high maternal antibody titer since they have been shown to be especially susceptible.

Staining of the basal ganglia not associated with isoimmunization has been described in connection with prematurity,^{1,10,19} in patients with spherocytic anemia,¹⁰ in those with congenital familial nonhemolytic jaundice,⁹ in newborn infants given large doses of vitamin K,^{4,8} in premature infants given sulfisoxazole (Gantisin) in the first five days of life,^{18,19} and even in the newborn infant with severe physiologic jaundice. The use of sulfonamides and salicylates may be hazardous in the newborn infant with hyperbilirubinemia because of the

ability of these substances to displace serum bilirubin from protein binding sites on serum protein and therefore allow its diffusion to other body compartments.^{11,21}

Kernicterus identical with that seen in human beings occurs naturally in a strain of jaundiced rats.¹⁵ The defect is the same in both species namely a lack of the conjugating glucuronyl transferase enzyme in the liver. In the animals as in human beings the administration of sulfonamides results in a lowering of the serum bilirubin and intensification of nervous signs and nuclear staining. Here too the suggested explanation is that kernicterus results from the competition between bilirubin and the sulfonamides for binding sites on the serum albumin so that unbound bilirubin becomes available for damage to nerve tissue.

Since the red cells of the premature infant may be susceptible to hemolysis with the development of jaundice in the infant drug therapy such as excessive doses of vitamin K and sulfonamides should be avoided unless a specific need exists.⁴

It has been estimated that approximately 5 to 15 per cent of all live born infants with erythroblastosis will develop kernicterus if untreated. About 70 per cent of babies with kernicterus will succumb within seven days of birth. Of the 30 per cent that survive the acute stage of cerebral symptoms neurologic sequelae are likely to develop at a later date. They constitute about 10 per cent of all cases of cerebral palsy.⁶ More specifically a fatal outcome is to be expected when signs of cerebral involvement develop on the second day and survival is more common when such signs appear on the fifth day.¹⁶ In the group that survive evidences of cerebral damage so frequently present consist of mild to severe spasticity, athetosis, auditory disturbances and defects of ocular movements.⁴

Intense jaundice without signs of kernicterus may be followed by slight mental impairment amounting to a reduction of the intelligence quotient of about 12 per cent.¹⁷ Deafness is a common sequel of kernicterus and should be investigated in all affected infants despite the absence of other signs of damage to the nervous system.

Children who have recovered from hemolytic disease may show green pigmentation of the deciduous teeth. It is of interest that an acute enamel hypoplasia of the deciduous teeth has been observed in patients with kernicterus following erythroblastosis.²²

Pathology. The pathologic changes reflect a number of factors: duration and intensity of the destruction of the red cells by maternal antibody in utero and in the first days of life; compensatory hematopoiesis; and effect of abnormal stresses upon the function and structure of immature tissues and organs.

In deeply jaundiced infants the entire brain may be pigmented. Staining is more intense with a brighter yellow color in the basal ganglia and other nuclei in the brain stem and medulla in the kernicteric infants. Ganglion cells degenerate and disappear leaving empty spaces.⁶ The mechanism of these changes is unknown but it may be related to inhibition by excessive bilirubin of oxygen uptake within the cells.¹

The liver and spleen are invariably enlarged. Histologically the most striking feature is widespread extramedullary hematopoiesis in these and other organs such as the pancreas, kidneys, adrenals, lymph nodes, thymus and placenta. Because of the persistent destruction of red blood cells there is a reversion to the embryonic type of blood formation. The bone marrow shows active hematopoiesis with a predominance of immature nucleated red blood cells. An enormous proliferation of normoblasts is particularly noticeable within the liver sinusoids compressing and displacing the liver parenchyma cells and resulting in pronounced degenerative changes. Many of the hepatic cells are vacuolated and in some cases are replaced by coarsened reticulum. Severely damaged livers show evidence of intrahepatic biliary obstruction with masses of bile pigment in the small bile ducts and in biliary canaliculi. Hemosiderosis may be found in the spleen and liver. Hypertrophy and hyperplasia of the pancreatic islet cells are striking. Petechiae even frank hemorrhages may be observed in the lungs. The adrenals reveal lipid degeneration of most of the cortical cells.⁶

Edema may be localized or severe and generalized as it is in infants with hydrops. In this condition extensive edema (especially of the face) varying degrees of maceration effusions into serous cavities and marked enlargement of the spleen and to a lesser degree of the liver are conspicuous findings. The placenta in cases of hydrops is edematous bulky and friable. The occurrence of erythroblasts in the pulmonary capillaries is the most important single diagnostic sign to be found in the macerated fetus.²⁷

Maternal Antibodies—Prenatal Testing. All pregnant women are initially typed for ABO group and the Rh factor. Whether or not the mother is immunized prenatal testing is carried out initially at the first visit or in the first trimester of pregnancy and is repeated after twenty eight to thirty weeks and at the end of pregnancy. When antibodies develop for the first time they are usually not detectable until about the fourth month of pregnancy. They may increase as gestation proceeds and are almost invariably detected by the thirty fifth week in an affected fetus. Should the termination of pregnancy be considered before term an additional test may be indicated. The absence of anti Rh antibodies in the mother's serum at this time usually indicates that the infant will not be affected by erythroblastosis due to the Rh factor.²⁸ On the other hand when antibodies are present at the beginning of pregnancy even in weak concentration the birth of an infant with erythroblastosis must be anticipated.

Maternal Anti Rh Titer. There is some general but not very close correlation between the type and concentration of antibodies and the clinical severity of the disease in the affected child. In general the disease tends to be milder when the predominant anti Rh antibodies are of the saline agglutinating type rather than the immune or incomplete form.

By and large higher titers of incomplete antibodies are associated with severe disease and a worse prognosis (1:32 to 1:64 and over). Exceptions to severe and fatal cases (stillbirths) with low titers and mild symptoms despite high titers have been noted.⁴⁶

Because of the risk of kernicterus a high maternal anti Rh titer (1:64 or higher) of incomplete antibodies has been regarded as a sufficient indication for

exchange transfusion immediately after birth in the infant with erythroblastosis regardless of the physical findings or hemoglobin level⁴³

A rising anti Rh titer indicates that the fetus is Rh positive. The antibodies derived from immunization with a previous Rh positive fetus persist during a subsequent pregnancy with an Rh negative infant (heterozygous father) and have been stated to actually increase in titer.⁷ This has been challenged by Wiener and co workers⁴⁴ who found that pregnancy with an Rh negative fetus and the birth of an Rh negative baby had no effect upon the Rh antibody titer of an Rh negative woman sensitized to the Rh factor.

In general if the father is heterozygous and if the maternal antibody titer remains constant the chances are fairly good that the infant will be Rh negative. A low titer that fails to change during pregnancy suggests no specific stimulation.

There is a great variability in the titer of the sensitized mother following delivery. It generally falls somewhat or may continue to be high for prolonged periods.

Effect of Previous Transfusions on the Mother Transplacental immunization can be detected in pregnancy when there is a history of blood transfusions or fetal death. Transfusions of Rh positive blood or intramuscular injections of Rh positive blood increases an Rh negative mother's susceptibility to immunization by an Rh positive fetus. Repeated transfusions are especially potent agents in producing a high degree of sensitization stillbirths and hydrops.

Immunization in the Rh Positive Mother and Infant At times there is no obvious incompatibility between mother and child both being Rh positive yet sensitization is indicated by neonatal hemolysis and a positive Coombs test. In the absence of ABO incompatibility consideration must be given to immunization with C, E, Kell and other less frequent factors. In such cases the husband's blood also should be examined regardless of his reaction to anti D (Rh) serum for his erythrocytes may possess a minor blood group antigen lacking in those of his wife.

Heterozygous and Homozygous Status of the Husband If the husband is Rh positive it is important in management to determine whether he is homozygous or heterozygous for the Rh factor. Although this may be reflected to some degree by the presence or absence of the Rh factor in previous offsprings appropriate tests will determine this with greater accuracy. The use of anti C, anti F, anti c and anti c provides a means of determining the husband's probable genotype. Since anti d serum is unavailable the diagnosis remains presumptive. In the heterozygous father the chances are 50 per cent that the offspring will be Rh positive or Rh negative. For the homozygous father (for D or Rh) it can be fairly safely predicted that all the children will be Rh positive.

Different Types of Antibodies Antibodies react under varying physical conditions. Some agglutinate red cells suspended in saline solution whereas others will only coat them. Both forms are found in the blood of mothers sensitized to Rh positive cells. The saline active or "complete" antibodies agglutinate red cells containing the Rh factor; they do not cross the placenta and hence are not involved in the causation of erythroblastosis. The second type will fail to agglutinate Rh positive cells when suspended in saline solution but will do so when

The liver and spleen are invariably enlarged. Histologically the most striking feature is widespread extramedullary hematopoiesis in these and other organs such as the pancreas, kidneys, adrenals, lymph nodes, thymus and placenta. Because of the persistent destruction of red blood cells there is a reversion to the embryonic type of blood formation. The bone marrow shows active hematopoiesis with a predominance of immature nucleated red blood cells. An enormous proliferation of normoblasts is particularly noticeable within the liver sinusoids, compressing and displacing the liver parenchyma cells and resulting in pronounced degenerative changes. Many of the hepatic cells are vacuolated and in some cases are replaced by coarsened reticulum. Severely damaged livers show evidence of intrahepatic biliary obstruction with masses of bile pigment in the small bile ducts and in biliary canaliculi. Hemosiderosis may be found in the spleen and liver. Hypertrophy and hyperplasia of the pancreatic islet cells are striking. Petechiae even frank hemorrhages may be observed in the lungs. The adrenals reveal lipid degeneration of most of the cortical cells.⁶

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Except for erythroblastosis due to anti A and anti B when the reaction is often weak or negative a weak reaction due to anti Rh is usually associated with clinically mild forms of the disease

Positive Coombs tests are obtained not only in patients with ABO and Rh erythroblastosis but also in those with idiopathic acquired hemolytic anemia acquired hemolytic anemias secondary to leukemia lymphosarcoma disseminated lupus erythematosus and thrombotic thrombocytopenic purpura

Indirect Coombs Test The indirect test is used to demonstrate circulating antibodies which coat but do not agglutinate cells suspended in saline solution In this in vitro test the serum in question is incubated with corresponding Rh positive red cells of a compatible ABO group Anti globulin serum is added to the incubated red cell suspension which has been thoroughly washed Readings for agglutination are made as in the direct test If the patient's serum contains incomplete or immune anti Rh antibodies they coat the test red cells and agglutination follows on the addition of Coombs serum The serum can be titrated by preliminary dilution with saline solution and made even more sensitive with the use of trypsinized test cells

Tests With Trypsinized Cells Saline suspended red cells are so altered by preliminary exposure to a trypsin solution as to give direct readings with incomplete or immune antibodies³ This test does not replace the indirect Coombs test for it fails to detect such rare antibodies as Duffy Kell and Kidd factors The method however is widely used and constitutes a valuable adjunct to tests employed for the detection of circulating antibodies

Elution The specificity of the antibody causing damage to the infant's erythrocytes in erythroblastosis can also be demonstrated by elution tests^{18, 24, 44} This is a method of releasing antibody from red cells which had adsorbed it The exact nature of the antibody involved may be determined through the use of the heat elution test on umbilical cord blood samples showing a positive Coombs test Eluates are prepared from erythrocytes of umbilical cord blood by preliminary washing of the red blood cells in cold buffered saline solution and later subjecting the suspension to 56° C. The eluates are tested for hemagglutination against known A or B red blood cells or positive cells of Rh Hr or other systems The test is particularly valuable when sensitization to antigens in both the Rh and the ABO systems is possible

Frequency of Blood Group Factors Causing Erythroblastosis Until recent years incompatibility with respect to the Rh factor (D) has accounted for the majority of cases of erythroblastosis A lowered incidence however is being noted due to the fact that many parents who have had one or two children with erythroblastosis are limiting the size of their families

Erythroblastosis due to A and B incompatibility is being recognized more widely since there is at present a greater alertness to its presence on the first day of life The frequency of erythroblastosis involving the A and B factors has been estimated as 1 per cent of all newborn infants as compared with 0.5 per cent with the Rh factor (Rh or D)⁴ Immunization with A is more frequently encountered than with B

Rh incompatible mating occurs in 13 per cent of all marriages Of incom

these cells are suspended in colloid medium such as plasma serum or albumin. These antibodies are variously termed incomplete univalent hyperimmune cryptagglutinoïd, glutinin immune and albumin. Of these incomplete and immune are most commonly used.

Blocking antibodies are a variety of "incomplete" agglutinins which fail to agglutinate test cells suspended in saline solution or in colloid media. They sensitize saline suspended cells by coating their surface. The antibody (globulin) coating will be detected with the use of an antihuman globulin (Coombs) serum which agglutinates sensitized cells (direct Coombs test). Many affected Rh positive (D or Rh⁺) infants appear to be Rh negative on typing, because the cells are heavily coated with incomplete antibodies. The most dependable test to determine the presence of such blocked or coated cells is the application of the direct Coombs test.

Naturally occurring antibodies such as anti A and anti B isagglutinins in persons with group O blood are those normally present throughout life which can be attributed to no known antigenic stimulus. Immune anti Rh antibodies are of pathologic and diagnostic significance and originate as a response to a specific stimulus during pregnancy and to transfusion.

Transmission of Antibodies Only the incomplete or immune form of anti Rh antibodies are capable of crossing the placenta and this transmission can occur at any early stage of pregnancy. When the incomplete and complete forms are present together in maternal serum only the incomplete form appears in the fetal serum.⁶ While there is some evidence that Rh antibodies are present in human milk and colostrum it is doubtful whether they can be absorbed even in the first days of life.⁷ Present practice has been to allow mothers to nurse their infants if they want to do so—certainly after the second day. Weaning infants with erythroblastosis because maternal milk contains antibodies is not justified.

Coombs Test (Antiglobulin Test) The Coombs test is a sensitive method of demonstrating the presence of an antibody adsorbed or bound to red cells. This test has come into common use since Coombs and associates⁸ found that serum from rabbits immunized with human globulin agglutinated human erythrocytes coated with Rh antibodies. Experience has shown this to be an invaluable method of detecting incomplete Rh antibodies. The essential component of the Coombs serum is the anti gamma globulin. There are two forms of the Coombs antihuman globulin test: the direct and indirect.

Direct Coombs Test The direct test is used to detect antibodies fixed to infants' cells *in vivo*. Antiglobulin serum is added to a suspension of cells which has been washed three times with saline solution. After the mixture is centrifuged it is observed for agglutination. Cells of the cord blood following delivery of an infant to an immunized woman are commonly used in the test to determine coating or sensitization while in utero. It is useful in confirmation of the typing of an infant presumably Rh negative. A positive Coombs test on such blood indicates that the red cells have been sensitized and that the infant is Rh positive. The earlier negative test was due to the blocking action of maternal antibody. A positive Coombs test establishes the diagnosis of erythroblastosis but is not in itself an indication for exchange transfusion.

moderately and severely involved infants to be followed by somewhat more severely affected infants.²⁹ Since a woman who has had one stillbirth on an Rh basis has about 4 chances in 5 of losing her next Rh positive fetus,²⁹ it would seem reasonable to advise premature induction of labor if the husband is homozygous.

Laboratory Findings—Blood The blood findings include the following features

Red Blood Cells The peripheral blood gives striking evidence of a hemolytic process with signs of active regeneration. The number of red cells, reticulocytes and hemoglobin concentration depend upon the severity of the disease and the ability of hematopoietic tissues to compensate for the destruction of red cells.

The hemoglobin and red cells may be normal but usually are moderately to markedly reduced at birth. The red cell count ranges from less than 2 million to as high as 5.5 million per cubic millimeter. The most prominent feature in the blood smear is the large number of nucleated red cells in every stage of maturation. This normoblastic outpouring reflects the accelerated regenerative activity of the bone marrow and extramedullary tissues. The red cells are predominantly macrocytic with slight anisocytosis and poikilocytosis. Spherocytosis is not a feature of the blood smear in Rh erythroblastosis as it is in erythroblastosis due to A-B incompatibility.

Although the number of normoblasts in the blood of the newborn infant does not exceed 10 per 100 white blood cells in the first two days of life, in the severely affected infant it may rise to 5 or 10 nucleated red cells for every white blood cell. In mild cases it is not increased above the normal. Normoblastemia also may occur in infants with congenital heart disease, in premature infants, in infants whose mothers have diabetes, in infants who have had fetal hemorrhage, and in those with anoxia.

Hemoglobin The hemoglobin varies from 5 gm per 100 ml to normal values of 15 to 18 gm. Infants with marked anemia are critically ill with edema, and those with congestive failure show a hemoglobin under 10 gm with a red cell count of 3 million or less. Although the hemoglobin concentration is normally measured in blood pricked from the heel, Mollison and Cutbush³⁰ have emphasized that it is obligatory to obtain the hemoglobin level from the cord blood. Cord blood hemoglobin serves as a more accurate index of the status of fetal blood and a more precise guide to treatment than the higher values obtained from venous blood and even the more elevated reading acquired from blood obtained by finger puncture. The reasons for these differences stem from the delayed typing of the umbilical cord blood, the passage of placental blood into the fetal circulation, and the rapid shift of plasma from blood vessels soon after birth. Thus Mollison and Cutbush cite an instance of a cord blood hemoglobin value of 12.8 gm per cent with venous and capillary blood samples of 15.4 and 18 gm, respectively. Such discrepancies are frequently responsible for confusion in determining the need for an exchange transfusion within the first twenty-four hours of life.

The normal cord blood hemoglobin of 16.6 gm (16.55 ± 1.5) reported by Mollison and Cutbush³ corresponds almost exactly with the values of 16.4 (16.4 ± 1.09) found in our laboratory. The value of 13.6 gm per cent

patible matings about 50 per cent of the Rh positive fathers will be heterozygous so that half of the offspring may be Rh negative and hence without disease.

Erythroblastosis due to the Rh factor in a population with about 15 per cent Rh negative persons occurs in 1 in 150 to 1 in 200 of all full term pregnancies. The fact that only 1 in every 20 to 26 full term incompatible pregnancies results in an infant with erythroblastosis emphasizes the inability of most Rh negative mothers to produce Rh antibodies.²¹ Even in Rh negative persons sensitized by transfusions with Rh positive blood there are many (about 10 per cent) who fail to develop antibodies even after a second transfusion or an incompatible pregnancy.

Anti D (Rh_0) antibodies often in combination with anti C or anti E account for most of the cases of erythroblastosis due to the Rh factor. Outside of the Rh factor and A and B factors which constitute the majority of cases c(hr') E(rh') Kell and a lesser number of blood factors outside of the Rh system mentioned previously (Chapter 6) account for the remaining 1 or 2 per cent of erythroblastosis.

The frequency of erythroblastosis is directly proportional to the frequency of the Rh negative persons in any given population. The incidence is less in Negroes in the United States with about 5 per cent Rh negative and is exceedingly rare in Chinese, Japanese and American Indians among whom only 1 per cent or less are Rh negative.²²

Prognostic Considerations and Family Patterns of Severity. The titer of maternal anti Rh constitutes a primary factor in determining the prognosis in the fetus in utero. If the anti Rh titer is less than 1:64 the chances are nearly 90 per cent that the baby will be in good condition at birth.⁴

Certain predictions can also be made with respect to the severity of the disease in successive siblings. The disease tends to be milder in the first affected infant than in those that follow. However the trend of increasing severity is not uniform in every family and mild cases follow severe cases even if the infant with the severe case is stillborn. About 5 to 10 per cent of first affected infants will be stillborn and of those born alive 40 per cent will not require treatment.⁴⁴ If the first sibling is mildly affected then those that follow are likely to be mildly affected and the chances of a subsequent stillbirth are relatively low (about 2 per cent).¹¹ If a previous baby was stillborn on an Rh basis there is about an 80 per cent chance that the next baby, if spontaneously delivered, will also be stillborn.¹¹

In another series it has been estimated that after a woman has had one stillbirth due to erythroblastosis the chances for the next Rh positive fetus are stillbirth 75 per cent, born alive but with extreme anemia 15 per cent, and born alive but with readily treatable disease 10 per cent. When a woman has had two stillbirths due to erythroblastosis the chances for the next Rh positive fetus are stillbirth 90 per cent, born alive but with extreme anemia 8 per cent, and born alive with readily treatable disease 2 per cent.

Greater prognostic accuracy can be obtained by referring to severity of the disease in the immediately preceding sibling. Thus there is a tendency for the very severely affected and stillborn infants to be followed by stillbirths and for

thrombocytopenia beyond the third or fourth day after an exchange transfusion with bleeding prompted a search for some other causative process. Congenital thrombocytopenic purpura with a failure of megakaryocytes or a pyogenic infection should be considered in such instances.⁴¹

Bilirubinemia Since kernicterus can be prevented in almost every case of erythroblastosis by keeping bilirubin levels below critical levels of 20 mg per 100 ml it is essential that bilirubin determinations be carried out from birth and frequently repeated during the first four or five days of life. Micromethods are now available⁴² which require only a small quantity of blood obtained from heel puncture. Another technique has been described in which the plasma heme pigments measured spectroscopically also serve as a reliable guide to the need for one or more exchange transfusions.⁴³ Bilirubin determinations however are more universally used.

The cord blood bilirubin levels in most normal infants are under 3 mg per 100 ml. The average is 2.2 mg (range 1.5 to 3.2) on the second day for the full term infant and approximately 11 mg (range 0.0 to 27) on the third to fourth day in the premature infant. Usually the bilirubin level begins to fall spontaneously between the second and third day in the full term infant and on the fourth or fifth day in the premature baby. The fall is more gradual in the premature infant and original cord values may not be reached until the tenth day or later.

A knowledge of these normal values serves as a background for the fluctuations in the infant with erythroblastosis. The cord blood bilirubin level of 3.5 and above in the affected infant is not much higher than that of the normal infant because of the clearing of this pigment in utero probably by the placenta. Of greater importance in management is the rate of rise of bilirubin as determined by periodic estimations. This ranges from 0.3 to 1.0 mg per hour depending upon the severity of the disease with the upper limit occurring in advanced disease. In some instances the rise is gradual over the first two days and accelerates on the third or fourth day.

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therefore represents the lower level of normal for cord blood. These authors gave values of 14.5 gm per cent for venous blood and slightly over 15.4 gm for capillary blood as the low limit of normal on the first day of life which should serve as a guide to management when cord blood determinations are not available. Thus the range of normal hemoglobin values in newborn infants³⁰⁻³¹ may be stated as follows: cord blood 13.6 to 19.6 gm per 100 ml; venous blood (first day) 14.5 to 22.5 gm per 100 ml; blood obtained by skin prick (first day) 15.4 to 22.8 gm per 100 ml.

Severity of erythroblastosis has been classified on the basis of cord hemoglobin concentration: mild disease 14 gm per 100 ml and above; moderately severe disease 11 to 13.5 gm; and severe disease less than 11 gm.

In erythroblastosis the concentration of fetal hemoglobin is significantly lower than normal whereas the concentration of adult hemoglobin is the same as in normal infants. There is no preferential destruction of fetal hemoglobin containing erythrocytes in this disease; rather there is a preferential regeneration of adult hemoglobin in response to hemolysis. In infants born with normal hemoglobin levels the capacity to synthesize adult hemoglobin in amounts greater than normal appears to be an important mechanism serving to prevent anemia.³²

Reticulocytes. The reticulocytes range above 6 per cent (upper limit of normal) in the infant with mild erythroblastosis and reach 40 to 50 per cent in those with severe disease. The reticulocyte count may also be increased when the cord blood hemoglobin is normal, indicating the ability of erythropoietic tissue to compensate adequately for the demands of hemolysis. A normal hemoglobin level therefore may be deceptive, and the need for repeated reticulocyte counts in such a case cannot be overemphasized as an index of underlying blood destruction. An infant with a normal hemoglobin level and an elevated reticulocyte percentage in the first two days of life may be in a precarious condition for in many cases the hemoglobin is known to drop precipitously on the third day with the development of severe disease and the need for urgent treatment. The reticulocytes are of great value therefore in assessing the regenerative process since they are often found in increased numbers when nucleated red cells are within normal limits.³³

Leukocytes. In infants with severe erythroblastosis the leukocytes vary from 15,000 to 30,000 per cubic millimeter. Higher counts have been recorded but these include large numbers of nucleated red cells. Myelocytes are common. Erythrocytes which have undergone phagocytosis by monocytes or neutrophils are occasionally found in blood smears.

Platelets. The platelet count may be normal or diminished in infants with severe erythroblastosis. Thrombocytopenia may be sufficiently marked to cause petechiae and purpura. Usually the thrombocytopenia parallels the degree of the hemolytic process.³⁴

PLATELET CHANGES FOLLOWING EXCHANGE TRANSFUSION. Desforges and O'Connell observed³⁵ low platelet levels several days after an exchange transfusion (as low as 50,000 per cubic millimeter and less in some cases) with a gradual rise beginning with the third day and restoration of normal values in about a week. Despite lowered values there was no bleeding tendency. The persistence of

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Although bilirubin is removed simultaneously with the red cells of the infant by exchange of plasma it frequently rebounds to approximately two thirds of pretransfusion levels because of pigment sequestered in the extravascular reservoirs. It has been demonstrated that bilirubin accumulates in the plasma during exchange transfusion even while it is being removed and must therefore enter the circulation from the tissues.⁹ The bilirubin withdrawn from tissue spaces is supplemented by that resulting from the breakdown of transfused blood especially if it has been stored for some time.⁴ Bilirubin is most effectively removed by a slow exchange and with large volumes of blood. Anti Rh antibodies are also inadequately removed by an exchange transfusion because of their wide distribution in extravascular spaces.

In infants with Rh erythroblastosis the immediate objective is to replace the vulnerable Rh positive cells which are the sources of bilirubin with the calculated amount of Rh negative cells. The concurrent aim is the removal of pigment from the intravascular and extravascular fluid compartments. This procedure is usually accomplished expeditiously within an hour or an hour and a half. In the deeply jaundiced patient in whom correction of the anemia may not be so important as the removal of bilirubin an interval of about one half to one hour may be permitted to elapse between each volume of the exchange. This delay will depend upon the condition of the infant and the general over all care in the treatment room.

The blood volume also can be adjusted by exchange transfusion especially in infants with cardiorespiratory embarrassment. Infants with cord blood hemoglobin levels of 8 gm per 100 ml or less are often distressed at birth and can be shown to have elevated venous pressure and often cardiac failure.¹⁰ One factor which may contribute to excessive blood volume and circulatory overload is the sudden passage of placental blood into the newborn infant at birth—hence the precaution of clamping the cord at the earliest moment. In this type of case every effort is made to reduce blood volume and venous pressure by establishing a deficit before beginning the exchange transfusion. The venous pressure is measured at intervals during the exchange and appropriate deficits are created as necessary. Artifacts in venous pressure readings do occur. If the pressure is unusually high the pulse and respiration will be increased. Therefore these should be used together with venous pressure readings as criteria for the need to establish a deficit. The reduction in blood volume and correction of the anemia by an exchange transfusion at birth may be life saving in the severely anemic infant.

Indications The indications for exchange transfusion reside in the demonstration by clinical and laboratory means that the disease in the infant is and may be expected to be of sufficient severity to lead to the afore mentioned complications. To a large extent the criteria for immediate exchange stem from clinical and laboratory evidence that a severe hemolytic process has been going on in utero. Thus clinical evidence of severe disease such as pallor, edema, poor respirations and hepatosplenomegaly constitute indications for immediate exchange (jaundice at birth is practically never seen). Laboratory evidence of a severe prenatal hemolytic process includes the presence of anemia at birth (as

Erythroblastosis Fetalis (Hemolytic Anemia of the Newborn Infant)—Treatment

Objectives of Treatment The treatment of the infant with erythroblastosis fetalis has three major aims: (1) the prevention or control of cardiac failure (the major cause of death within the first twenty-four hours), (2) the prevention or control of bilirubinemia in an effort to prevent the occurrence of kernicterus (the major cause of death after forty-eight hours), and (3) the control of anemia (this is almost automatically associated with the first two aims). It will be seen that except in the infant with severe anemia and cardiac failure, treatment is primarily oriented toward keeping the blood bilirubin low rather than toward achieving a high hemoglobin level.

To date the only effective therapy in controlling the aforementioned complications is exchange transfusion. In evaluating an infant for exchange transfusion, therefore, the physician must decide whether or not the disease state in the infant is severe enough to place him in danger of cardiac failure or subsequent hyperbilirubinemia of sufficient magnitude to cause kernicterus. Optimally the decision concerning replacement transfusion should be made as soon as possible, since cardiac deaths often occur within the first few hours of life, and since the prevention of bilirubinemia is much more efficient than the removal of the pigment once accumulation has been permitted to occur.

Treatment The mainstay of treatment is the exchange transfusion, but its successful achievement depends upon many correlated items of procedure.

Exchange Transfusions

Rationale Exchange transfusions accomplish the removal of all but a small fraction of the infant's own destroyable and sensitized Rh-positive red cells and their replacement by an adequate concentration of normal nonsensitized Rh-negative cells. Unless the infant's red cells are replaced, they will continue to be destroyed by the maternal anti-Rh antibodies which persist in the circulation for four to eight weeks after birth. With a two-volume exchange (twice the blood volume of the infant), 85 to 90 per cent of the sensitized red cells are recovered.

- (b) Hepatosplenomegaly
- (c) Poor respiration
- (d) Jaundice
- (e) Edema--with or without definitive signs of cardiac failure

2 Laboratory evidence of severe hemolytic disease

- (a) Cord hemoglobin of 14 gm or below (less than 14.5 gm venous blood or 15 gm capillary blood)
- (b) Reticulocytosis over 6 per cent
- (c) Normoblastosis greater than 10 per 100 white blood cells

It should be remembered however that a low cord hemoglobin level may coexist with a low cord bilirubin level (below 3 mg) hence the greater importance of the former as a criterion for exchange at birth.

3 Factors favoring development of kernicterus

- (a) History of severe disease stillbirth or kernicterus in a previous sibling
- (b) Prematurity
- (c) Maternal titer greater than 1:64

Indications for initial late exchange or repeated exchange

- 1 Continued signs of cardiac failure or severe illness
- 2 Indirect bilirubin levels rising at greater than 0.5 mg per cent per hour reaching 7 mg at six hours 10 mg at twelve hours and approaching 20 mg at any time

These indications may be restated as follows

An exchange transfusion is performed at birth in an infant with a positive Coombs test who also presents one of the following abnormalities

- 1 Clinical evidence of severe disease at birth with or without cardiac failure
- 2 Cord hemoglobin level of 14 gm and below
- 3 History of at least moderately severe disease of kernicterus or of stillbirth in a previous sibling
- 4 Prematurity except in the presence of the mildest disease
- 5 Cord bilirubin levels above 4 mg per 100 ml

A maternal titer of 1:64 or more should alert the pediatrician to the possible need for exchange transfusions. However in institutions where facilities for sequential bilirubin determinations are limited exchange transfusions should be carried out at birth at this titer. Wheeler and Ambuel have pointed out that 80 per cent of babies with a cord blood level above 4 mg per 100 ml eventually require an exchange transfusion whereas only 20 per cent of those with cord bilirubin below this level needed treatment. These criteria for replacement transfusions are acceptable in most clinics for severe disease is usually anticipated if the infant is allowed to go untreated.

If exchange transfusion is not performed at birth the bilirubin level is determined every four to six hours during the first day and every six to twelve hours on the second and third days. Exchange transfusions are then given if

The cord hemoglobin level at which exchange transfusions are recommended varies for the full term infant from below 11 gm per cent to below 15.5 gm per cent in different clinics. The level of 14 gm per cent of hemoglobin is derived from the mean cord blood hemoglobin level obtained in a series of 133 normal infants in our clinic ($16.5 \text{ gm} \pm 1.5 \text{ gm}$).

determined by a low hemoglobin level in the the cord blood) elevation of cord bilirubin (above 3 mg per cent) and manifestations of marked attempts at blood regeneration (normoblastosis reticulocytosis)

The baby threatened by cardiac failure always has severe anemia. On the other hand however an infant with good powers of regeneration in utero may be born with a nearly normal hemoglobin concentration and look clinically well but may still be in severe danger of subsequent hyperbilirubinemia. This is due to the fact that the hemolytic process will proceed unabated postnatally but that the mechanism for excreting bilirubin will be inadequate. In utero clearance of bilirubin is apparently carried out mainly by the placenta. Postnatally however bilirubin excretion is dependent upon the infant's liver function and the activation of a hepatic enzyme system which converts the insoluble indirect bilirubin to a soluble bilirubin diglucuronide (direct bilirubin) which may then be excreted. Development of efficient bilirubin excretion usually requires three to four days in a full term baby and longer in a premature infant. It is essential therefore to detect early those infants with a severe hemolytic process and to prevent the occurrence of bilirubinemia by removing the Rh positive antibody coated cells and replacing them with Rh negative cells.

In addition to the criteria described there are other indications for immediate exchange based upon the special susceptibility of certain infants to kernicterus. These include premature infants, infants in a family with previous stillbirth and kernicterus, and infants born of mothers with very high antenatal antibody titers.

It has been well established that the incidence of kernicterus may be reduced to extremely low levels if the serum bilirubin is kept below 20 mg per cent. In the mature infant with erythroblastosis the likelihood of kernicterus has been estimated as about 1 in 10 for an indirect serum bilirubin of 20 to 25 mg per cent, about 1 in 5 for 25 to 30 mg per cent and about 1 in 2 over 30 mg per cent.¹¹

In the small premature or in any premature infant whose condition is poor it may be necessary to postpone the exchange transfusion because of the risk involved. The known susceptibility of such infants to kernicterus aggravates the problem. Under these circumstances careful observation is necessary, successive bilirubin determinations should be evaluated and consultation with other physicians may be necessary before treatment is undertaken.

Before treatment is contemplated it is important to ascertain that the major portion of the bilirubin is of the indirect reacting type. Whereas this pigment is associated with brain damage, increased concentration of direct bilirubin indicates obstruction or an inflammatory process in the biliary system. An exchange transfusion for a high direct reacting bilirubin would be purposeless.

Indications for exchange transfusion may be summarized as follows:

Indications for immediate exchange at birth

A summing that the direct Coombs test is positive, the indications for an immediate exchange are as follows:

1. Clinical signs

(a) Pallor

juncture (that is in the older healthy infant with bilirubin levels fluctuating narrowly about 20 mg per 100 ml) the decision must ultimately be left to the clinical judgment of the attending physician who has full knowledge of both the hazard of hyperbilirubinemia and the potential risk of an exchange transfusion.

PREMATURITY. In a premature infant in whom the maternal titer is high that is reaching levels of 1:64 and in whom the cord blood bilirubin level is in the vicinity of 3 mg per 100 ml with hemoglobin levels of about 13 gm it may be necessary to postpone exchange transfusion if the infant's condition would be jeopardized by the procedure. During the ensuing period successive bilirubin determinations are made and evaluated before treatment is undertaken. This is not a simple matter and no unequivocal measurement can be employed to fill all contingencies. The peculiar problems presented by the premature infant with erythroblastosis are similar to those in connection with exchange transfusion in physiologic hyperbilirubinemia of full term and premature infants and are discussed further later in the chapter. Parents should be informed that an exchange transfusion carries a small but definite mortality per se on which are superimposed the handicaps of prematurity. Prematurity of two weeks or more constitutes a compelling indication for the prophylactic employment of exchange transfusions when the indications are otherwise equivocal.⁹

INDUCTION OF LABOR. In the infant with very severe disease the chances of survival diminish with the period of gestation. It has been estimated that a woman who has had one stillbirth caused by Rh erythroblastosis has about 4 chances in 5 of losing her next Rh positive fetus if it remains in utero until term.⁸ Most intrauterine deaths occur before the thirty-seventh week of gestation. Induction of labor accordingly has been recommended at or after thirty-seven weeks of gestation in sensitized Rh negative women when conditions are obstetrically favorable and the fetus is of good size and is viable. Specifically the indications are history of a severely affected infant, hydrops or stillborn baby, a homozygous father, or a rising maternal antibody titer or one that has already reached 1:64. Cesarean section is exceptionally advised if the mother's condition is unfavorable for induction of labor, usually after a trial of pitocin and ruptured membranes. Based on a repetitive history of stillborn infants and a rising maternal titer, induction of labor has been recommended as early as thirty-two weeks in selected cases.¹⁰ The present trend, however, is for early delivery for sensitized mothers in whom the prognosis for the infant seems good but at a time closer to term.⁶⁰

Since the baby may be premature and may have already sustained the effects of blood destruction, exchange transfusion is prepared for in advance of delivery with a supply of type O Rh negative blood at hand compatible with the mother's serum. The earlier fear of a high incidence of kernicterus in the slightly premature baby has now been dispelled by its known prevention with the use of multiple exchange transfusions.

Whole blood versus sedimented red cells—the protective action of albumin. Although whole blood has been almost universally used in replacement, sedimented blood has its advocates.^{2, 43} The advantage of sedimented red cells is

bilirubin rises to 7 mg. at six hours and 11 mg. or more in the first twelve hours even if the hemoglobin level is above 15 gm. (peripheral blood). From one to four exchange transfusions may be required to keep the level of bilirubin below 20 mg. per 100 ml.

It should be emphasized that after the initial cord blood hemoglobin level is determined subsequent hemoglobin levels serve no purpose in deciding on the need for exchange transfusions to prevent kernicterus. The later hemoglobin determinations merely indicate the need for correcting the anemia which can be accomplished by small transfusions of packed cells. The bilirubin level however remains the only available index of the need for an exchange transfusion.⁴⁰

Specific problems: Specific problems relating to exchange transfusion include age at time of treatment, prematurity, and induction of labor.

AGE AT TIME OF TREATMENT: The late development of kernicterus usually can be avoided by early vigorous treatment when the trend of bilirubin levels is definitely upward during the first twenty-four to forty-eight hours. Following this policy, the problem of an initial transfusion beyond the second day of life is usually never pressing. Boggs, for instance, has reported a cure in 53 of a group of 56 infants born to women who had had a previously affected infant provided the infant was first seen before the age of 12 hours.

Individual problems arise however after the first seventy-two to ninety-six hours at which time the infant may be expected to begin excreting bilirubin momentarily. The question as to what age it is no longer beneficial to perform an exchange transfusion in the face of hyperbilirubinemia is most difficult to answer. No time limit in days can be set beyond which hyperbilirubinemia is no longer dangerous in the full-term infant and in the premature infant it is even more difficult. According to one point of view,⁴⁷ exchange transfusions need no longer be considered after the fifth day in a full-term infant or after the sixth day in a premature infant if hyperbilirubinemia has not yet developed. According to another view, no exchange transfusions are necessary in the full-term infant after the age of 5 days or in the premature infant after the age of 10 days.⁴¹

On the other hand, the need for an exchange transfusion is not readily assessed if the bilirubin level which has risen slowly in the first days reaches levels somewhat below 20 mg. per 100 ml. and if on the fourth day after birth the infant is vigorous, takes its feedings well, and shows no nervous system irritability. In such infants serial bilirubin levels must be observed through the first week of life if necessary. High bilirubin levels cannot be ignored at any specific number of days following birth. Exchange transfusions are indicated in the infant at 7 days of age, for example, if the bilirubin is in excessive concentration.⁴² Patients have even been described in whom the early signs of kernicterus have been reversed by one or more exchange transfusions. Except for the established bilirubin level of 20 mg. per 100 ml., there is no way of knowing at which of the lower levels a particular infant is in jeopardy of nervous system involvement. Infants with bilirubin levels greatly in excess of 20 mg. have been known to be free of nervous system irritation whereas some with much lower levels have had nervous system involvement. Since there is no uniform opinion at this

Sterile pack

Plastic catheters (polyvinyl)
 2 infant feeding tubes sizes #K31 and #K32
 Sterile disposable plastic tubing (polyethylene) PE 190/S12 and PE 90/S12†
 3 three-way stopcocks
 15 cm stainless steel rule
 3 metal basins
 1 cut-down set
 1 transfusion set

1 L. syringes (10 mL)
 Rubber tubing (to fit the stopcocks)
 Umbilical cord tape
 Sterile drapes dressing to include 1 circumcision sheet and 2 gowns
 Sterile jar 8 by 3 inches with Zephiran chloride solution
 Sterile test tube with catgut with curved needle (plain 3-0)

Unsterile equipment

Operating board with facilities for heating hot water bottle aquamatic h. pad
 Blood warming apparatus‡
 Oxygen
 Suction

Gravity pole
 Waste bucket
 2 Ace bandages (2 inch width)
 1 circumcision board

Also available should be heparin solution 10 per cent calcium gluconate solution antibiotics and specimen tubes for coagulated and uncoagulated blood. Blood for exchange transfusions should be as fresh as possible (no older than four days) to avoid the risk of dangerous increases of potassium in the plasma and should be properly warmed before administration. The blood should never be placed in a water bath *above* 37 °C because of the danger of causing hemolysis.

The procedure for the exchange transfusion is as follows

- 1 On a fairly large sterile field all sterile equipment to be used should be laid out so that it will be within easy reach of the operator
- 2 The tubing should be rinsed inside and out with saline solution and smoothed
- 3 The three way stopcocks are then fastened in tandem and attached to a 20 ml syringe and to the catheter. The discharge tube is attached to the proximal stopcock so that removal of the syringe or emptying of the tube will not allow air to fall into the distal stopcock. The transfusion recipient set is attached to the distal stopcock.
- 4 One of the basins should be filled with sterile saline solution. The other should be filled with saline solution to which heparin solution has been added (1:300)
- 5 The syringes should all be rinsed with the heparin saline mixture
- 6 The baby should be completely immobilized on a Y board leaving the abdomen exposed. The immobilization should allow for the provision of external heat suction and oxygen. The abdominal wall should be prepared by cleansing and draping as for a laparotomy

†Pharmaseal Laboratories Glendale Calif

‡Intramed c. Clay Adams Inc. New York, N. Y.

§Corman Rupp Industries Inc. Belleville Ohio

¶Hospital Instruments Co. Scotia N. Y.

that the hemoglobin concentration is less likely to fall to levels requiring further transfusion. In this method approximately 100 to 150 ml of the supernatant ACD plasma layer is removed from a bottle of previously stored Rh negative blood.*

One of the primary objectives of exchange transfusions is the removal of maximal amounts of bilirubin already present in the plasma and that which enters the circulation from tissue depots during the exchange. Another indirect advantage is that plasma protein especially albumin which serves as a vehicle for binding bilirubin is supplied in larger quantity by whole blood than by the restricted amounts present in sedimented blood. Transfusion with whole blood receives renewed strong support from the demonstration of a protective effect of albumin against the development of kernicterus.¹¹⁻¹⁵ The use of albumin solutions as an adjunct to exchange transfusions to bind bilirubin in extravascular spaces appears to have merit.^{3,16}

Whole blood and packed red cells have been used in combination in a single procedure with the latter employed for about one third of the exchange transfusion.¹⁷ The use of whole versus sedimented cells deserves further clinical and experimental evaluation because of increasing interest in the possible advantages of the latter. Sedimented red cells have already found a place in exchange transfusions in infants with profound anemia who are often in cardiac failure and show a high venous pressure.⁵ In this case a one to one and a half volume exchange is usually advisable because of the precarious state of the infant. Except for this emergency, whole blood is the preferred medium in exchange transfusions especially in infants with elevated or rising bilirubin levels.⁶

For the present whole blood is recommended in exchange transfusions especially in infants with elevated and rising bilirubin levels.

Estimation of the amount of blood to be used. The infant's blood volume should be estimated on the basis of 85 ml per kilogram of body weight. The total amount to be used equals twice the blood volume. The amount of blood actually exchanged by alternate withdrawal and introduction equals the total minus amount initially withdrawn. The remaining donor blood (equal to the amount withdrawn initially) may be carefully given as a terminal transfusion only if the venous pressure is normal.

Example—3 kg infant

Blood volume 255 ml
Total blood ordered 510 ml
Initial withdrawal 30 ml
Exchange 480 ml

Procedure. Whenever possible the exchange transfusion should be performed by catheterization of the umbilical vein. The operator must be prepared however to do a cut down if necessary.

The necessary equipment should be completely assembled before the baby is exposed.

*From a mixture of 500 ml of blood and 120 ml of ACD (acid citrate dextrose) anti-coagulant.

should be obtained for various determinations (Coombs test total protein hematocrit hemoglobin bilirubin etc.)

- 15 The catheter should be withdrawn the cord carefully tied and a sterile dressing applied to the area (If a second exchange is contemplated a saline dressing should be used.)
- 16 Following the exchange prophylactic antibiotics are given 100 000 units of penicillin and 30 to 40 mg per kilogram streptomycin intramuscularly in divided doses every twelve hours for four to seven days.

If the clinical condition of the infant indicates that a second exchange will probably be required the catheter may be filled with sterile heparinized saline solution plugged off and left in the vein covered with a sterile dressing. A catheter should not be left in place more than eight to twelve hours. Any prolonged stay may lead to infection. Actually it is rather easy to reinsert a catheter into the vein previously used.

The rapid movement of bilirubin into the plasma during or immediately following an exchange transfusion the so called rebound phenomenon has prompted an interruption of the procedure for approximately one hour between the first and second volumes of blood. Provided the infant is maintained in good condition this modification has proved very effective in cases in which the removal of bilirubin is the prime objective. It is hoped that this device will reduce the need for repeated exchanges.

Saphenous vein method In the method described by Wiener and co workers³ the infant is bled from the radial artery while blood is introduced into the internal saphenous vein. Although this procedure constitutes an effective method of exchange transfusion the intermittent substitution method utilizing the umbilical vein without the need for skin incision is the one in common use. If the umbilical vein is definitely unavailable the femoral or saphenous vein is used instead.

Heparinized blood Heparin instead of citrate has been employed as an anti-coagulant for exchange transfusions^{4, 37, 48} but not as yet on a widespread scale. Heparinized blood has many advantages: the blood is necessarily warm and not refrigerated since it is used within a short period after being drawn; it avoids citrate and potassium intoxication; frequent calcium gluconate injections are unnecessary; and the blood is more concentrated with higher hemoglobin levels since dilution by acid citrate dextrose mixtures is avoided. The obvious disadvantages are that appropriate donors must be immediately available since heparinized blood keeps poorly and that there is the rare possibility of a coexisting bleeding tendency in the infant. Heparin is used in a dosage of 15 mg per 500 ml of blood. To avoid the danger of hemorrhage 15 mg of protamine sulfate solution is administered via the umbilical catheter at the end of the procedure.³ This step is not always carried out.

Unexplained death Exchange transfusions are not without their dangers and unexpected fatalities occur. It has been estimated that the frequency is about 1 per cent of replacements. Cardiac arrest has been attributed to hyperpotassemia hypocalcemia citrate toxicity and acidosis. In any one case it is al-

- 7 When everything is ready and after all equipment has been checked the procedure should be started. Aspiration of the infant's stomach is a useful preliminary procedure.
- 8 The umbilical cord should be cut squarely 1 cm from the skin margin. The umbilical vein should be identified (it is the largest of the three vessels and at the 12 o'clock position). Any visible blood clots should be removed with forceps. One edge of the vein should be picked up with mosquito forceps. The catheter should then be inserted into the lumen of the vein for 1 centimeter or so. Suction should be applied through the syringe and the cannula withdrawn so as to remove any deeper blood clots. After making certain that the catheter has been rinsed clear it should be gently inserted into the vein until blood is obtained. If difficulty is encountered slight cradled traction should be made on the vein and the catheter inserted and withdrawn until the proper channel has been found. (The introduction of a catheter more than 7.5 cm beyond skin surface may place the tip within the heart.) When the catheter is in the right position umbilical cord tape should be placed around the cord and cannula to provide an airtight seal. As previously stated artifacts of venous pressure readings must be kept in mind and will be recognized if pulse and respirations are normal.
- 9 The system should then be rinsed through with saline solution, the stopcocks disconnected from the catheter and a venous pressure reading should be obtained by measuring the height of the column in the catheter against the steel rule placed just above the umbilicus or at the xyphoid process. (Normal venous pressure is 4 to 8 cm.)
- 10 The proper amount of blood should be withdrawn before any blood is administered. Specimens both coagulated and uncoagulated should be obtained for various determinations. For full term infants with high hemoglobin values and without cardiac failure 20 to 40 ml of blood may be withdrawn before any blood is given. This deficit improves the efficiency of the exchange.
- 11 The infant should then be given 20 ml of the donor blood. Thereafter the exchange is continued with the alternate withdrawal and introduction of equal amounts of blood. For the average baby 10 ml increments are quite satisfactory.
- 12 Throughout the procedure care must be taken to keep syringes and tubing well rinsed to prevent clotting. If the infant struggles or cries it may be an indication of an increased venous pressure. This should be checked before proceeding. A written record of the exact amount of blood introduced and withdrawn should be kept.
- 13 The infant should then be given 1 ml of 10 per cent calcium gluconate solution after each 100 ml of blood introduced. This should be diluted in saline solution and given over a period of several minutes with careful auscultation of heart sounds and rate during the administration.
- 14 When the required amount of blood has been exchanged a final sample

Anemia in the Previously Treated Infant One or more supplementary transfusions with packed cells (10 ml per kilogram) are given before discharge from the hospital to raise the hemoglobin to 10 to 12 gm per 100 ml. Since anti Rh antibody persists for at least four to eight weeks following birth, weekly hemoglobin determinations are necessary during this period. Transfusions of packed cells are given in the clinic in the first two or three weeks following discharge from the hospital when the hemoglobin level falls below 8 gm per 100 ml.

After this period transfusions are usually unnecessary, particularly if a reticulocytosis appears unless the 8 gm concentration of hemoglobin is associated with illness. Transfusions above this level may suppress innate hemoglobin and red cell synthesis which is usually in progress between the sixth and eighth weeks of life. Hematinics are without value in the prevention or treatment of anemia at this time.

Hyman and Sturgeon⁴ have shown that during the convalescent period of erythroblastosis fetalis there is often an increase in total hemoglobin despite a fall in hemoglobin levels. This discrepancy is explained on the basis of growth since a simple calculation of blood volume will reveal that the infant is indeed regenerating hemoglobin which is not reflected in the reduced peripheral hemoglobin concentration.

In severe erythroblastosis, however, anemia may persist for several weeks following discharge from the hospital and is stated to exceed that which is expected from continuing hemolysis after successful replacement has been achieved or from an expanding blood volume due to growth of the infant. This form of anemia has been attributed to bone marrow hypoplasia as a complication of severe hemolytic anemia in the newborn infant¹⁴ from suppression of the inherent blood formation due to the known inhibitory effects of transfusion¹⁵ or from antibody specifically directed against the red cell in the fetus and extending into the neonatal period.¹⁶

Comprehensive bone marrow examinations, however, do not explain the continuing anemia following the acute hemolytic period on the basis of a decrease in the number of normoblasts in the bone marrow.¹⁷ On the contrary, serial examinations indicate that the percentage of normoblasts in the bone marrow is increased in proportion to the anemia and that a true regenerative state appears to be a rare occurrence. The persistent anemia following the acute hemolytic stage of erythroblastosis probably represents a diminished reactivity of the bone marrow which may be more severe than is normally present.

Anemia in the Previously Untreated Infant It is important to watch those babies closely after discharge who have not had previous exchange transfusions because their anemia was mild (10 to 11 gm per 100 ml) during the hospital stay. In an occasional case a precipitous drop in the hemoglobin occurs in the second and third weeks of life. Death from severe and progressive anemia may result from excessive blood destruction coupled with inadequate compensatory blood formation. Transfusions to maintain adequate hemoglobin levels are urgent in these infants.

Rupture of the Spleen Splenic rupture is a rare complication of erythroblastosis occurring regardless of therapy. Many factors have been incriminated: extramedullary hematopoiesis, increased phagocytosis, weakening of the supportive structures, and the tendency to hemorrhage.^{23,44}

Management of the Infant With Erythroblastosis When a sensitized mother enters the hospital for delivery of her baby, the pediatric service and the blood bank should be alerted immediately by the obstetric service. At the time of de-

most impossible to single out the causative factor. Cases of cardiac arrest during exchange transfusion have been reported which were successfully treated by thoracotomy and cardiac massage.^{18, 36}

Miscellaneous Treatment The following are some of the auxiliary measures suggested for treatment of patients with erythroblastosis.

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The use of ACTH as a substitute for exchange transfusions has met with failure despite transient increases in hemoglobin levels. On the other hand there is evidence that as an adjunct to exchange transfusion therapy it reduces the need for repeating exchange transfusions. Klingberg and Jones recommended the use of ACTH in a dosage of 125 mg every twelve hours by intramuscular injection for five to seven days until signs of excess hemolysis and bilirubinemia occur. More carefully controlled investigation is needed before consideration can be given to its use.

Glucuronic Acid The administration of glucuronic acid by oral and intravenous routes has been advocated as a means of converting the insoluble indirect or free bilirubin which accumulates in the blood of infants with erythroblastosis and is associated with kernicterus to the water soluble direct bilirubin which is excreted by the liver. Although lowering of indirect bilirubin has been reported by one group³² it has not been substantiated by others. This approach bypasses the essential enzymatic nature of bilirubin glucuronide formation. Uridine diphosphate glucuronic acid is the source of the glucuronic acid of conjugated bilirubin and is transferred to bilirubin by an active enzyme system. Since free glucuronic acid does not enter into the sequence of reactions the biochemical basis of this therapy is doubtful. Furthermore it has been shown by animal experiments that kernicterus may develop despite a depression of serum bilirubin when glucuronic acid is given.³³ It has been suggested that this agent produce a redistribution of bilirubin from the blood to other body compartments with a potential increase in concentration in central nervous system tissue. Claims for the efficacy of glucuronic acid were finally withdrawn when it was shown that the same effects on the concentration of bilirubin in the serum resulted from comparable amounts of glucose and saline solution given intravenously.

Results of Treatment With the reservations imposed by the uneven character of the severity of cases over the years the drop in overall mortality and incidence of kernicterus has nevertheless been obvious. At The New York Hospital the improvement is reflected in the drop in mortality from 35.8 per cent in the pre-exchange period (1932 to 1941) to 8.9 per cent in the exchange transfusion period (1947 to 1955). The liberal use of exchange transfusions has resulted in a striking drop in the incidence of kernicterus from 25 per cent in 25 infants with erythroblastosis in 1946 to a total absence of this complication in 31 infants in 1955.

Anemia in the Previously Treated Infant One or more supplementary transfusions with packed cells (10 ml per kilogram) are given before discharge from the hospital to raise the hemoglobin to 10 to 12 gm per 100 ml. Since anti Rh antibody persists for at least four to eight weeks following birth weekly hemoglobin determinations are necessary during this period. Transfusions of packed cells are given in the clinic in the first two or three weeks following discharge from the hospital when the hemoglobin level falls below 8 gm per 100 ml.

After this period transfusions are usually unnecessary, particularly if a reticulocytosis appears unless the 8 gm concentration of hemoglobin is associated with illness. Transfusions above this level may suppress innate hemoglobin and red cell synthesis which is usually in progress between the sixth and eighth weeks of life. Hematinics are without value in the prevention or treatment of anemia at this time.

Hyman and Sturgeon have shown that during the convalescent period of erythroblastosis fetalis there is often an increase in total hemoglobin despite a fall in hemoglobin levels. This discrepancy is explained on the basis of growth since a simple calculation of blood volume will reveal that the infant is indeed regenerating hemoglobin which is not reflected in the reduced peripheral hemoglobin concentration.

In severe erythroblastosis, however, anemia may persist for several weeks following discharge from the hospital and is stated to exceed that which is expected from continuing hemolysis after successful replacement has been achieved or from an expanding blood volume due to growth of the infant. This form of anemia has been attributed to bone marrow hypoplasia as a complication of severe hemolytic anemia in the newborn infant from suppression of the inherent blood formation due to the known inhibitory effects of transfusion or from antibody specifically directed against the red cells in the fetus and extending into the neonatal period.⁴⁸

Comprehensive bone marrow examinations, however, do not explain the continuing anemia following the acute hemolytic period on the basis of a decrease in the number of normoblasts in the bone marrow. On the contrary serial examinations indicate that the percentage of normoblasts in the bone marrow is increased in proportion to the anemia and that a true regenerative state appears to be a rare occurrence. The persistent anemia following the acute hemolytic stage of erythroblastosis probably represents a diminished reactivity of the bone marrow which may be more severe than is normally present.

Anemia in the Previously Untreated Infant It is important to watch those babies closely after discharge who have not had previous exchange transfusions because their anemia was mild (10 to 11 gm per 100 ml) during the hospital stay. In an occasional case a precipitous drop in the hemoglobin occurs in the second and third weeks of life. Death from severe and progressive anemia may result from excessive blood destruction coupled with inadequate compensatory blood formation. Transfusions to maintain adequate hemoglobin levels are urgent in these infants.

Rupture of the Spleen Splenic rupture is a rare complication of erythroblastosis occurring regardless of therapy. Many factors have been incriminated: extramedullary hematopoiesis, increased phagocytosis, weakening of the supportive structures, and the tendency to hemorrhage.^{45, 44}

Management of the Infant With Erythroblastosis When a sensitized mother enters the hospital for delivery of her baby the pediatric service and the blood bank should be alerted immediately by the obstetric service. At the time of de-

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The volume of blood used in exchange transfusion is best referred to in terms of multiples of the infant's own blood volume. Thus for a child with an estimated blood volume of 250 ml. the commonly used 500 ml. replacement transfusion is a two volume replacement. Such an exchange provides an 87 per cent replacement of the blood, but the exchange of the red blood cells is dependent upon the hematocrit of the donor blood and upon the infant's initial hematocrit. The higher the donor hematocrit and the lower the infant's initial hematocrit the higher the percentage of Rh negative cells which will be present in the infant's circulation at the end of the transfusion.

For an exchange of 500 ml. of blood with the average hematocrit of 36 per cent the hematocrit of Rh negative cells at the end of the transfusion (and this will be the infant's ultimate hematocrit when all of his own Rh positive cells have been destroyed) will be 32 per cent. The percentage of Rh negative cells will range from 82 per cent for an infant with an initial hematocrit of 50 per cent to 96 per cent for an infant with an initial hematocrit of 10 per cent. The size of the transfusion can also be determined according to the nomogram of Veall and Mollison which provide about 90 per cent replacement with donor blood and a final total hematocrit of 50 per cent in the infant.

Treatment of the Infant in Cardiac Failure In most cases the signs of congestive failure (pallor, dyspnea, restlessness, grunting respirations, cyanosis) will be found in severely anemic infants. Blood is withdrawn cautiously, initially the amounts depending upon the elevation of venous pressure and general condition of the infant. In infants with congestive failure usually 30 to 60 ml. are withdrawn, however as much as one third of the infant's blood volume may be withdrawn. Sedimented cells (150 ml. of supernatant ACD plasma removed) are recommended for the anemic infant with heart failure. Replacement is carried out slowly and less than the calculated amount is given—approximately one to one and a half volumes of blood. Administration of digitalis has been found unnecessary. Antibiotics should not be administered through the umbilical vein for fear of an adverse reaction through an effect directly on the cardiac musculature.

Exchange Transfusion in Physiologic Hyperbilirubinemia of the Full Term and Premature Infant In exceptional cases the indirect reacting bilirubin level in the normal full term infant and especially in the premature infant reaches critical levels comparable to those in infants affected with Rh and ABO erythroblastosis. Neonatal jaundice not caused by blood group incompatibility is considerably more common in premature than in mature infants. Exposure to concentrations of bilirubin of 20 mg. and above carries the same hazard of kernicterus especially in the premature infant. It may be that the peculiar susceptibility of the premature infant to kernicterus is due to the greater permeability of bilirubin through the blood cerebrospinal barrier in the premature infant than in the full term infant.¹⁰ Jaundice can be controlled by exchange transfusions as it is in the infant with erythroblastosis. The exchange however must be carried out more slowly and re-exchange may be required since the objective is the removal of bilirubin from the circulation rather than the removal of vulnerable cells as in cases of erythroblastosis. The transfused blood should be of the same group and Rh subtype as the infant.

livery a member of the pediatric staff should be in attendance. Observation should be made of the color of the vernix and of the amniotic fluid. They may be stained yellow in erythroblastosis fetalis. When the baby is delivered the cord should be clamped immediately. This is done for several reasons. There is no advantage to the baby in a transfusion of antibody coated red blood cells and circulating antibody. A great many of the more severely affected infants have an elevated venous pressure and would do better with a smaller blood volume. It is estimated that the child may receive up to 100 ml of blood from the placenta.

It is of the greatest importance that the umbilical cord be cut so as to allow a segment at least three inches long between the umbilicus and the tie. The cord stump should be wrapped in gauze soaked in saline solution and great care must be taken to keep this tissue from becoming dried out.

The child should be examined for manifestations of hemolytic disease: jaundice (rarely immediately at birth), pallor, petechiae, edema and hepatosplenomegaly. At the same time several blood specimens should be carefully collected from the placental portion of the cord.

A 5 to 10 ml specimen of oxalated umbilical venous blood is examined for hemoglobin, ABO group, Rh type and direct antiglobulin*. In the event of a positive Coombs test, a serum bilirubin determination and a complete blood count including reticulocytes and normoblasts are carried out. When there is any suspicion of erythroblastosis and the Coombs test has been erroneously reported as negative, complete blood tests should nevertheless be carried out until the Coombs test is verified; this should be done without delay. When cord blood is not available, venous blood or blood obtained by pricking the heel can be used for the initial tests. The peripheral blood may be more suitable for study of red and white cell morphology and reticulocytes.

After the preliminary studies have been performed the subsequent course may be decided. It must first be determined of course whether or not the child is erythroblastic.

Group specific ABO Rh negative blood is usually given. When a severely affected infant is anticipated, group O Rh negative blood should be available before delivery. In any case the blood used should always be compatible with the mother's serum. The prospective donor's red blood cells also should be cross matched with the serum of the infant's mother and the compatibility should be confirmed by the indirect Coombs technique. The mother's serum not only contains much more anti Rh antibody than the infant's serum but may contain antibodies other than Rh (O). It is only by such tests that other incompatibilities between mother's and infant's blood can be completely eliminated. Group O Rh negative blood should be selected with low anti A and anti B titers and A and B substances (Witebsky) should be added.

*At The New York Hospital 5 ml of clotted blood is sent to the blood bank for routine typing and the Coombs test. The remainder of the blood specimen is saved for cross matching. From 5 to 10 ml of oxalated umbilical blood is set aside for use of the pediatrician who carries out the serum bilirubin determination and the complete blood count including reticulocytes.

appear to have outnumbered those due to the Rh factor. With few exceptions the cases are confined to A or B offspring of group O mothers with the A factor predominating.

The restriction of ABO erythroblastosis to 5 per cent of incompatible matings is due to several mechanisms by which fetal erythrocytes are protected from the action of maternal antibodies. The most important probably relate to the neutralization or fixation of potentially harmful maternal antibody by specific A or B blood group substances located in fetal and placental tissues.^{1, 2}

Clinical Features. In about 50 per cent of cases the first born is usually affected in contrast to Rh incompatibility in which the first born escapes unless the mother has had previous transfusions. The occurrence of A B erythroblastosis is therefore unanticipated unless a previous pregnancy has established the possibility of incompatibility from these blood factors.

An important distinguishing feature of A B erythroblastosis is the development of jaundice within the first twenty-four hours of life—hence the designation “*icterus precox*.”¹ Therefore jaundice appearing before 24 hours of age almost invariably means erythroblastosis due to A or B factor. This must not be confused with physiologic jaundice which appears on the second or third day when jaundice due to A B incompatibility is frequently at its peak. In the severe type of A B disease the icterus increases in intensity from the first day and may be as pronounced as corresponding crises of Rh incompatibility. Anemia at birth is rare and if it is present it is usually mild. Splenomegaly is slight to absent. Stillbirth and hydrops are rare and the prognosis for succeeding pregnancies is excellent. A tendency toward early recovery is characteristic. Hyperbilirubinemia can occur and if untreated results in kernicterus as in cases of Rh incompatibility. The occurrence in the first born has been attributed among other causes to diverse A and B antigen-like substances contained in material used for immunization procedures.

Laboratory Findings—Blood. The hemoglobin level and red blood cell count are usually either normal or slightly reduced. In most infants hemoglobin levels usually range from 15 to 18 gm per 100 ml in the first days of life. There is seldom a substantial fall in the hemoglobin level during the course of the disease. Increased normoblasts are less common than in Rh disease. The white blood cell count is moderately elevated. It is of interest that infants with erythroblastosis due to anti A antibody belong to subgroup A₁.³⁰

The reticulocyte count is an essential test of special significance in A B erythroblastosis. As in patients with Rh incompatibility reticulocytosis indicates overactivity of the bone marrow to compensate for the destruction of red cells of a shortened life span. A normal hemoglobin level and reticulocytosis (over 10 to 17 per cent) indicate excessive blood destruction and are usually accompanied by rising bilirubin levels.

The most characteristic hematologic finding in A B erythroblastosis is the presence of microspherocytosis as revealed by the blood smear. This feature is in contrast with the almost uniform macrocytosis present in Rh disease. Spherocytosis is best observed in the first days of life. As would be expected there is an associated increase in osmotic and mechanical fragility. Differentiation

It is still too early to judge the efficacy of exchange transfusions in this group of infants because of limited experience. The problem is particularly trying in the premature infant whose physical condition does not warrant exposure to a prolonged technical procedure. Under these circumstances evaluation of serial bilirubin determinations provides the only means of controlling the situation. It may be necessary to defer treatment even with a rising bilirubin in the case of a small premature infant whose physical condition is precarious. In general one cannot yet be too dogmatic as to the exact bilirubin level at which an exchange transfusion should be undertaken in these infants.¹⁴ Clinical judgment in the individual case is needed and in the group of premature and full term infants a rigid yardstick cannot as yet be applied until a great deal more information is obtained.

It is of interest that although available evidence has emphasized the susceptibility of premature infants with hyperbilirubinemia to kernicterus, Holman¹⁵ pointed out the difficulties of assigning critical levels over which the nonimmunized premature infant requires treatment by exchange transfusion. For instance in his series of fourteen such premature infants with bilirubin concentrations exceeding 18 mg per 100 ml there was no clinical evidence of kernicterus in the neonatal period. One explanation for this inconsistency was the preponderance of Negro infants who are notably more mature than white infants of the same weight. Undoubtedly other auxiliary factors play a role in determining the ultimate concentration of bilirubin or are actually involved in producing kernicterus. Prolonged follow up is necessary to determine minor and major neurologic sequelae in this group of infants. The complexity of this subject is reflected in the comments on Holman's paper.¹⁶ Crosse and colleagues emphasize that replacement should be performed promptly in premature infants if the serum level of indirect bilirubin is over 18 mg per 100 ml or at a lower level if the bilirubin is rising rapidly. Meyer¹⁷ cautions that there might even be justification for a prophylactic exchange transfusion with a rapidly rising bilirubin before levels of 18 and 20 mg are reached. News and Norron¹⁸ would give transfusions to those infants with a serum bilirubin of 30 mg and below if they become lethargic, irritable, difficult to feed, or if there is a rapid rise of serum bilirubin on the third or fourth day. After reviewing their cases of hyperbilirubinemia without blood group incompatibility, Mores and co-workers¹⁹ claim that prophylactic exchange transfusion in full term infants is useless in premature infants; it is theoretically unfounded and its practical value has not been proved, not even by those performing it. In general the trend seems to be to give exchange transfusions to affected infants with the reservation that critical levels cannot be stated categorically. (For additional comments on physiologic hyperbilirubinemia see Chapter III.)

Exchange Transfusions as a Treatment of Poisonings The removal of toxic substances from the blood and tissues of children in acute poisoning has been achieved by exchange transfusion. The procedure has been successfully used in children with a wide variety of poisonings including barbiturates, boric acid,²⁰ methyl salicylate poisoning,²¹ and ferrous sulfate poisoning.⁴

ABO Erythroblastosis In recent years incompatibility within the ABO blood group system has been established as a common cause of erythroblastosis. The failure to recognize ABO disease in the past was due to the fact that frequently it was so mild that it was commonly overlooked. From the 20 to 25 per cent of heterospecific pregnancies in which fetal red cells possess an A or B factor not present in the mother's blood, a substantial number of cases of erythroblastosis develop. In approximately 95 per cent of these infants the blood type of the mother has been group O; in the others A or B factor. In recent years these

thin in blood samples from infants 24 to 144 hours of age. At The New York Hospital a record of the maternal major blood group is made available to the pediatrician during pregnancy. Blood typing and the Coombs test are performed in cord specimens of all infants of group O mothers. If a positive Coombs test is found, a hemoglobin determination, reticulocyte count and blood smear are carried out to look for nucleated red blood cells and spherocytes in an effort to establish the presence of active hemolytic disease in the infant.

Maternal serum in cases of A B erythroblastosis contains natural saline active antibodies anti A and anti B which are normally present in group O individuals. Only if the titer is excessively high (over 1:1024 against A agglutinogens and 1:512 against B agglutinogens) may it help in diagnosis.¹⁰

Mothers of affected infants also possess hyperimmune or incomplete anti A and anti B antibodies which sensitize the red cells of the fetus. The presence of immune antibodies in the blood of the mother with affected children can be demonstrated by their failure to be neutralized with soluble A and B group substances. This is the basis of a test for the titration of anti A and anti B antibodies after partial neutralization of the naturally occurring antibodies.¹⁰ Maternal serum containing these immune antibodies is capable of hemolyzing the infant's cells *in vitro*.¹⁰ Added complement is sometimes necessary to demonstrate this property. The immune bodies reach their peak during the first ten days postpartum and remain steady for the next four to six weeks. After that the titer slowly diminishes slowly in titer.¹⁰

Spontaneous agglutination of the infant's coated red cells termed *conglutination* can be demonstrated in A B erythroblastosis. Conglutination refers to the tendency of thick suspensions of sensitized or coated red cells (with anti A, anti B or Rh immune antibodies) to clump spontaneously in protein media such as adult serum, plasma and bovine albumin and in colloidal media such as gum acacia.¹⁰

One of the most reliable serologic methods for the diagnosis of A B erythroblastosis is the demonstration of free antibody in the infant's plasma capable of agglutinating adult erythrocytes of the same blood group as the patient.⁹ The presence of such homologous antibody is presumably derived from the transplacental passage of excess maternal antibody into the fetal circulation. In addition to these features the red blood cells of the affected infant clump spontaneously in plasma or colloid media.

Relation of ABO Compatibility and Rh Immunization. It has been pointed out that erythroblastosis due to the Rh factor occurs more frequently when ABO compatibility exists between mother and child.¹⁰ In one series¹¹ ABO compatibility between the mother and child was found to be present in 80 per cent of an unselected obstetric population in contrast to 95 per cent in a group of sensitized Rh negative women who bore infants afflicted with hemolytic disease.

Wiener⁴ ascribed the protective action of a heterospecific pregnancy on the basis of competition of antigens. Since both A and B are better antigens than Rh in man, the antigenicity of the weaker antigen may be suppressed. It seems more likely that the destruction of A and B cells leaves fewer red cells for Rh sensitization. However, exceptions have been reported in which erythroblastosis resulted from combined effects of Rh and hyperimmune anti A antibodies found in the mother.¹ Neutralized group O Rh negative blood is the recommended treatment.

from hereditary spherocytosis is sometimes difficult. The absence of a positive Coombs test and the presence of the disease in other members of the family are important features in the diagnosis of hereditary spherocytosis.

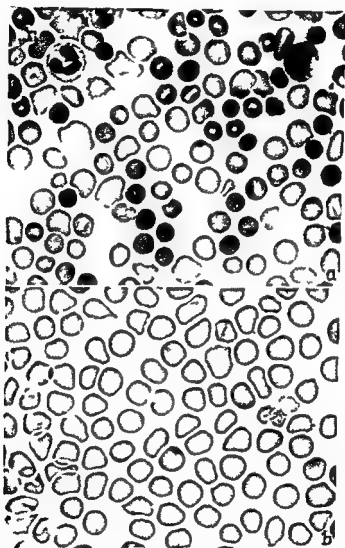


Fig 3 A Microspherycytosis in A-B hemolytic disease. B Uniform macrocytosis in Rh hemolytic disease (From Levine P, Vogel J P and Rosenfield R E *Advances in Pediatrics* vol 6 Chicago 1953 Year Book Publishers Inc.)

SEROLOGIC FINDINGS Without the use of refined methods the Coombs test with infant's cells (direct antiglobulin test) is generally negative or weakly positive in A or B incompatibility. With specialized techniques however the Coombs test will be positive in as high as 90 per cent of cases of ABO erythroblastosis. A strongly positive test is more likely to be observed in cord blood specimens.

must be left to the clinical judgment of the attending physician who is aware both of the sequelae of nervous system damage and of the potential hazard of an exchange transfusion

In the anemic infant in whom the level of bilirubin is low or moderate and not rising small transfusions of packed group O cells of compatible Rh type are given in the first days of life

The blood used in all cases in the exchange transfusion should be fresh group O of the appropriate Rh type in which anti A and anti B antibodies have been neutralized with 10 ml of blood group specific substances A and B (Witebsky substances) If possible group O blood with low a and b⁺ isoagglutinins is preferable As in cases of Rh sensitization re exchange may be necessary if hyperbilirubinemia persists exceeds original levels and shows evidence of rising to 20 mg per cent

As for subsequent pregnancies there is no fear of increasing severity as there is with Rh sensitization On the other hand since A B incompatibilities occur in at least 20 per cent of pregnancies it is a good plan to be alert to the recurrence of the disease However there is no evidence that antibody titers caused by heterospecific pregnancies ordinarily persist or affect unfavorably the outcome of future pregnancies ²⁰

Differential Diagnosis The following conditions must be excluded physiologic jaundice hereditary spherocytosis congenital nonspherocytic hemolytic anemia neonatal hepatitis toxoplasmosis cytomegalic inclusion disease congenital syphilis and bacterial sepsis in the newborn infant (see Chapter 8) Jaundice should be controlled by exchange transfusion in patients with those conditions in which bilirubin of the indirect reacting type is increased before critical levels of hyperbilirubinemia appear and kernicterus threatens These include jaundiced infants with hereditary spherocytosis and congenital nonspherocytic hemolytic anemia The same consideration applies to newborn infants especially premature ones in whom physiologic jaundice is intense and whose blood shows excessive bilirubin concentrations ² The differential diagnosis of jaundice in the neonatal period is given in greater detail in Chapter 8

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Treatment It must be emphasized that the proper indication for exchange transfusion in infants with ABO erythroblastosis is the degree of bilirubinemia and not the anemia. The prevention of kernicterus due to hyperbilirubinemia therefore constitutes the major objective in management of A B erythroblastosis as in Rh disease. Exchange transfusion is performed and repeated if necessary to keep the level of indirect serum bilirubin below 20 mg per 100 ml. Although the majority of infants with A B erythroblastosis are mildly affected and require no treatment they nevertheless bear scrupulous watching. About 20 to 30 per cent of infants with A and B incompatibility who develop icterus within the first thirty-six hours of life will need treatment. This compares with an estimated 80 to 90 per cent of Rh positive infants who were born to sensitized mothers and who require one or more exchanges.⁴⁰

A level of 10 mg on the first day of life suggests the possibility of higher levels in the next few days whereas the same level on the second or third day indicates a more benign course.⁸ A reticulocytosis over 10 per cent and a rapidly rising bilirubin level of more than 0.5 mg per cent per hour constitute indications for treatment. All infants with A B erythroblastosis should be closely observed during the first two days of life with measurement of daily hemoglobin and frequent serum bilirubin levels. With increasing jaundice bilirubin levels may be required every six to eight hours. If the bilirubin exceeds 10 mg per 100 ml in the first twelve to twenty-four hours an exchange transfusion is required. Deepening jaundice with a rise in bilirubin approaching levels of 20 mg on the second day of life also necessitates treatment.

Because jaundice is frequently mild and is overlooked in the first days of life the pediatrician is often confronted with unexpected hyperbilirubinemia on the third or fourth day. To avoid this contingency the ABO blood group and Rh typing of the mother and infant should be conspicuously placed on the chart and susceptible infants kept under scrutiny. Any infant who is jaundiced before 24 hours of age who has an indirect serum bilirubin level exceeding 10 mg per 100 ml in the first twenty-four hours and who is incompatible with the mother for A and B groups may be presumed to have erythroblastosis and should be treated.

The management of situations in which the bilirubin level approximates 20 mg on the third and fourth days of life when jaundice has gone unnoticed for the first forty-eight to seventy-two hours presents some difficulty. In these infants the rise in the concentration of bilirubin has probably been gradual. There is no unanimity of opinion as to treatment in these circumstances. Exchange transfusion in this group has not always been considered necessary because of the gradual rise in the concentration of serum bilirubin which eventually did not greatly exceed 20 mg per 100 ml.⁴ So many borderline cases of ABO erythroblastosis are encountered especially on the third and fourth day of life that it is difficult to abide by hard and fast rules. It is hoped that liver function will be established at ninety-six hours and that bilirubin levels will begin to drop. Unfortunately this does not always occur. Many regard it safe to postpone treatment for an additional day in a full-term infant who is in good clinical condition and who is taking feedings well if the bilirubin slightly exceeds 20 mg on the fourth day. As in cases of Rh erythroblastosis the ultimate decision in each case

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Anemias—General Considerations

Anemia may be defined as a condition in which the concentration of hemoglobin or the number of red blood cells either singly or in combination are reduced below normal. The volume of packed red blood cells per 100 ml of blood measured by the hematocrit undergoes a simultaneous but not always parallel reduction. The physiologic defect caused by the anemia is a decrease in the oxygen carrying capacity of the blood and a reduction in the oxygen available to the tissues. Usually anemia is said to exist when the hemoglobin content of the blood falls below normal.

Classification. On an etiologic basis anemia results from an increased loss or destruction of red blood cells or a decreased rate of production. Blood loss is due to acute or chronic hemorrhage and excessive hemolysis from intracorporeal defects or extracorporeal factors. Impaired hemoglobin and red cell formation are due to a deficiency of substances required for their synthesis. Defects of the red cells may be congenital or acquired but in either case their shortened life span frequently results in an anemia due to a failure of red cell production to keep pace with red cell destruction. Erythropoiesis also may be depressed by toxic chemical or physical agents by space occupying or infiltrative lesions of the bone marrow or by unrecognized causes. It will be noted that the classifications may overlap. For example anemia may result from a combination of excessive destruction of erythrocytes with intracorporeal defects as occurs in patients with hereditary spherocytosis and the hereditary hemoglobinopathies with inadequate compensatory erythropoiesis. (For the hemolytic anemias see Chapter 15 for the hereditary hemoglobinopathies see Chapter 16.)

Following is a classification of the anemias based on etiology.

1. Blood loss—acute and chronic hemorrhage
2. Excessive blood destruction
 - A. Intracorporeal or intrinsic defects usually hereditary
 - (1) Hereditary spherocytosis thalassemia sickle cell anemia the hereditary hemoglobinopathies
 - (2) Hereditary nonspherocytic anemia and elliptocytosis
 - (3) Hemolytic anemia due to enzyme deficiencies

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 - (3) Hemolytic anemia due to enzyme deficiencies

- III Extracorporeal factors
 - (1) Factors due to antibodies and isogglutinins
 - (2) Symptomatic hemolytic anemias
 - (3) Lederer's anemia march hemoglobinuria paroxysmal cold hemoglobinuria
 - (4) Miscellaneous causes such as infections and chemical and physical agents—hypersplenism
- 3 Decreased or impaired production
 - A Deficiency of substances required for hemoglobin and red cell formation iron vitamin B₁₂ folic acid ascorbic acid protein
 - B Depression or inhibition of bone marrow
 - (1) Infection chemicals physical agents metabolic products
 - (2) Idiopathic depression and aplasia with or without congenital anomalies
 - C Mechanical interference and replacement by abnormal cells leukemia Hodgkins disease malignancies
 - D Miscellaneous infection renal failure hypothyroidism

Orientation The anemias constitute the major segment of the blood disorders of infancy and childhood. Anemia is a syndrome of multiple etiology whose origins are frequently difficult to discern for reasons inherent in the developmental processes of the pediatric period. Many of the anemias undoubtedly are conditioned by factors operative in fetal life dating from the critical first months of gestation when the primary blood cell elements are established. Others are influenced by the postnatal physiologic and anatomic changes which originate within and outside of the hematopoietic system. Still others represent inherited inborn errors of metabolism such as the primaquine sensitive group of the hemolytic anemias. The conditioning factors influencing the blood picture in the neonatal period and early infancy are listed below. * Each of these items is discussed elsewhere in the text.

- 1 The results of maternal immunization by fetal blood factors
- 2 Bleeding from the placental surface (abruptio placentae placenta previa and other complications of delivery)
- 3 Fetal hemorrhage into maternal circulation
- 4 Anemia from congenital bleeding disorders overt or obscure hemorrhage
- 5 Fluctuations in blood volume as related to early or late tying of the cord
- 6 Substitution of adult for fetal hemoglobin
- 7 The regenerative phase of erythropoiesis which characterizes the early months of life
- 8 The possibility that a blood dyscrasia may represent a congenital or developmental defect
- 9 The effects of prematurity
- 10 The effects of rapid body growth
- 11 Tendency for the hematopoietic system to react excessively to a stimulus reactivation of extramedullary foci of hematopoiesis
- 12 Inadequate fetal stores of iron from maternal deficiency of this mineral
- 13 Hemolytic anemia from maternal transmission of ingested drugs or chemical compounds

Diagnosis The problems of diagnosis in the infant and young child are complicated by the fact that the anemias tend to develop insidiously so that the

complete hematologic picture with its specific criteria may be slow in emerging. Despite these apparent difficulties it is possible with minimal laboratory equipment to arrive at a diagnosis by the judicious appraisal of data derived from a variety of sources such as the following:

- 1 History and physical examination
- 2 Basic blood studies: complete blood count, reticulocytes, interpretation of blood smear
- 3 Comparison with normal range of blood values for each age period
- 4 Etiologic classification
 - A Blood loss
 - B Excessive blood destruction and hemolysis of extracorporeal and intracorporeal origin
 - C Bone marrow depression or infiltration
 - D Deficiency of building materials for hemoglobin or red cell formation
- 5 Reference to age periods of most frequent occurrence
- 6 Bone marrow examination
- 7 Roentgenographic examination
- 8 Study of hereditary factors
- 9 Therapeutic response to iron, folic acid, vitamin B₁₂, liver, transfusion
- 10 Specialized laboratory procedures
 - A Essential
 - (1) Stool guaiac test
 - (2) Serum bilirubin
 - (3) Blood urea nitrogen
 - (4) Coombs test
 - (5) Red cell fragility
 - (6) Hemoglobin electrophoresis
 - B Helpful
 - (1) Gastric acidity
 - (2) Stool urobilin
 - (3) Serum iron and latent iron binding capacity
 - (4) Bone marrow iron

The large number of items included in the list illustrates the wide scope of available information and the simpler technical procedures upon which the diagnosis is based. However, an insight into the nature of the anemia actually requires that relatively few of these topics be probed. Under ordinary circumstances a thorough history, physical examination and basic blood studies in conjunction with a simple classification based on etiology are usually sufficient to arrive at a correct diagnosis.

History and Physical Examination. Interrogation along broad lines of causation should include the following: any change of behavior pattern such as irritability, anorexia, inactivity, fatigability, onset of pallor, prematurity, rate of weight gain, quantity of milk ingested and attitude toward solid food. Inquiry should also extend into a history of exacerbations of pallor and jaundice, hematemesis, loss of blood from the bowel, infection with animal parasites, allergy, ingestion of drugs or household products known to depress hematopoiesis or to cause he-

molysis exposure to radiation frequency of respiratory infection skeletal pain joint swellings renal disease and purpura

Physical examination should include scrutiny of such diverse features as pallor jaundice skin pigmentation enlargement of lymph nodes and spleen petechiae and purpura In iron deficiency anemia acute leukemia and severe loss of blood the skin has a waxy whiteness Cardiac enlargement and signs of cardiac failure may be present A loud systolic murmur best heard at the apex and less commonly over the pulmonary area is frequently regarded as being of organic origin before the underlying severe anemia is discovered These murmurs are of hemic origin and usually disappear following transfusions In patients with advanced Cooley's anemia the facies have a characteristic appearance In this disease as well as in other conditions requiring multiple transfusions such as aplastic anemia and pure red cell anemia pallor is replaced by dark pigmentation and bronzing of the skin In patients in whom hemosiderosis is present and at times coexists with hemochromatosis sexual retardation is noted Increasing jaundice from the first day of life differentiates erythroblastosis fetalis from its slower development in icterus neonatorum Eye ground and nervous system changes are to be looked for in patients with sickle cell anemia Leg ulcers occur rarely in sickle cell anemia in children

Splenomegaly is most noticeable in infants with disorders attended by blood destruction such as erythroblastosis hereditary spherocytosis acquired hemolytic anemia and severe Cooley's anemia The spleen is occasionally enlarged in patients with iron deficiency anemia and those with megaloblastic anemia of infancy It is not palpable in infants with aplastic hypoplastic or pure red cell anemia It should also be remembered that a spleen palpable about 1 to 3 cm below the costal margin is present in a substantial number of otherwise normal infants and children The soft edge of the spleen in these children is in contrast with the hard edge found in patients with pathologic conditions Enlargement of the liver is occasionally found in patients with congestive heart failure and in those with sudden severe anemia from any cause It is often enlarged in patients with the hemolytic anemias especially in those receiving frequent transfusions and who have developed hemosiderosis and hemochromatosis

Except for the anemia associated with infection or with leukemia lymphadenopathy is not a conspicuous feature of anemia Enlarged cervical nodes frequently associated with a palpable spleen occur in the child with anemia due to repeated infections of the upper respiratory tract The superficial lymph nodes may be slightly enlarged in patients with severe Cooley's anemia and sickle cell anemia but not in those with the other intrinsic anemias A generalized lymphadenopathy in the presence of a refractory anemia and leukopenia should arouse suspicion of leukemia

Age Incidence in Relation to Diagnosis When infection which is responsible for the major number of anemias in the pediatric age group is excluded as an etiologic factor the remaining hematologic disorders can be grouped for orientation according to the age periods of their most frequent incidence newborn infancy and childhood

In the newborn infant the anemia is usually due to erythroblastosis fetalis If

this can be eliminated blood loss is to be considered. Acute fetal blood loss during labor and delivery frequently resulting in posthemorrhagic shock may be due to fetal bleeding from the placenta; occult transplacental loss of fetal blood into the maternal circulation or rupture of a normal or shortened umbilical cord.⁶

Anemia due to iron deficiency occurs most commonly between 6 months and 2 years of age especially in the rapidly growing infant in the premature infant and in any infant whose dietary iron is restricted. Iron deficiency anemia also occurs during infancy due to chronic gastrointestinal bleeding from embryonic structural defects such as diverticula. Megaloblastic anemia of infancy occurs chiefly between the ages of 2 and 18 months. Pure red cell (aregenerative) anemia is usually apparent at the age of 2 to 3 months or later in the first year. When the infant is at approximately 11 months to 1 year of age the clinical and hematologic features of severe Cooley's anemia, sickle cell anemia and hereditary spherocytosis are sufficiently conspicuous to be diagnosed. They can be distinguished even earlier in suspected cases because of a familial background.

Aplastic anemia, hypoplastic anemia and Fanconi's anemia syndrome (aplastic anemia combined with multiple congenital anomalies) usually manifest their complete clinical and hematologic features after 3 years of age. At about this time too Banti's disease and other disorders are encountered which are associated with splenomegaly and varying degrees of a hypersplenic blood picture such as that seen in Gaucher's disease. It must be emphasized that the onset of the blood disorders first recognized in older infants and young children precedes by a variable period the time when the distinguishing features are fully established and unequivocal.

Normal Blood Values in Infancy and Childhood Because of developmental changes anemias in infancy and childhood must necessarily be interpreted in the light of the contemporary hematologic framework within which they are encountered. The range of normal values for the respective age periods which serve as a basis for evaluating an anemia are summarized in Table 5. A knowledge of the values for hemoglobin and red cells at birth and in the early newborn period are essential for an awareness of an anemia due to erythroblastosis or blood loss.

For comparative purposes the average hemoglobin level on the first day of life may be regarded as 20 gm per 100 ml of blood with values ranging from 18 to 22 gm and the red cell count as 5,500,000 per cubic millimeter with a range from 5 to 6 million. During the first two years of life the normal hemoglobin content ranges from approximately 10 to 12.5 gm per 100 ml of blood with an average value of 11 gm and between 5 and 10 years of age from 13 to 13.5 gm; during puberty it rises gradually to the adult value of 14.5 gm per 100 ml. A count of 4 million red cells per cubic millimeter of blood may be regarded as the lower limit of normal for the older infant and child. In normal newborn infants the number of nucleated red cells in the first few days of life ranges from 3 to 10 per 100 white blood cells.

Volume of Packed Red Cells (Hematocrit) In all anemias the measurement of the volume of packed red blood cells by the hematocrit constitutes an important and essential guide for diagnosis and therapy. This determination reflects

the total mass of cells in a unit volume of blood and has proved to be of fundamental value in the study of all anemias in which considerable alterations occur

*Table 5 Normal Blood Values Significant in Diagnosis of Anemias in Infancy and Childhood**

Hemoglobin	
1st day	20 gm (18 to 22 gm)
2 wk	17 gm
1st and 2nd yr	11 gm (10 to 12.5 gm)
3 to 5 yr	12.5 to 13 gm
5 to 10 yr	13 to 13.5 gm
10 yr	14.5 gm
Red blood cells	
1st day	5 500 000 (5 to 6 million)
2nd wk	5 000 000
Older infant and child	4 000 000 per cubic millimeter lower limit of normal
Nucleated red cells	
Average—3 to 10 per 100 white cells (birth to 4 days of life)	
Reticulocytes	
0.5 to 1.5 per cent (6 per cent upper limit of normal—from birth to 4 days of life)	
(Below 0.5 per cent in aplastic and hypoplastic anemia; increased in hemolytic anemias; in deficiency anemia rise from low to high levels with treatment)	
Volume of packed red cells (hematocrit)	
Infants 1 mo. to 2 yr	34 per cent
Children 2 yr. to 12 yr	36 per cent
Older children	40 per cent
Serum bilirubin	
Newborn full term infants	2 to 8 mg. per cent
Newborn premature infants	1 to 15 mg. per cent
(Values given for both full term and premature newborn infants are the low values at birth rising to maximum during first week of life)	
Normal infants and children	Under 1 mg. per cent
(Hemolytic anemias—elevated total bilirubin predominantly indirect fraction)	
Fragility test	
Normal range	0.425 to 0.325 per cent sodium chloride
(Increased fragility in hereditary spherocytosis and in some cases of acute hemolytic anemia; decreased fragility in sickle cell anemia, thalassemia [major and minor] and in iron deficiency anemia)	

*From Smith C. H. *Anemias in Infancy and Childhood*. Diagnostic and Therapeutic Considerations. Bull. New York Acad. Med. 30:155, 1954.

in size shape and thickness of the red blood cells. It has the advantage of being reliable and least subject to error in quantitative interpretation. In patients with iron deficiency anemia for instance the extremely low hematocrit reflects the state of anemia more accurately than does the moderate reduction or even normal value in the number of red blood cells. Also in the asymptomatic person with the trait of thalassemia the volume of packed red cells frequently remains at the same level in spite of wide fluctuations in the number of red cells.

Because of the variations noted in available studies it is difficult to state the optimum packed cell volume for different age groups. As a working basis a hematocrit of 34 per cent may be regarded as the lower limit of normal in infants after the first month of life 36 per cent from 2 to 12 years of age and 40 per cent in older children.

Reticulocyte Count and Stain The reticulocyte count reflects the state of activity of the bone marrow hence this determination provides a useful guide in the diagnosis of both deficiency and hemolytic anemias and in gauging the response to treatment. Normally from 0.5 to 1.5 per cent of the red blood cells are reticulated levels below 0.5 per cent represent inactive bone marrow. A persistent depression in the percentage of the reticulocytes in spite of treatment occurs in patients with aplastic and hypoplastic anemia. In those with anemia due to a deficiency a reticulocyte response from previously low levels follows appropriate treatment. In patients with the hemolytic anemias the values for reticulocytes are constantly elevated and the high levels which are maintained prior to and without treatment represent intensified bone marrow regeneration. In such cases the Coombs test will be positive in infants with erythroblastosis fetalis and acquired hemolytic anemia. A negative test prompts a search for the diagnosis among the hereditary hemoglobinopathies primaquine sensitive hemolytic anemias nonspherocytic anemia and hereditary spherocytosis.

Reticulocyte stain A glass slide is cleaned and polished. Two or three drops of a saturated alcohol solution of brilliant cresyl blue are put near one end of the slide and allowed to dry. Heavy streaks of precipitated stain are removed by rubbing with lens paper.

A small drop of blood is placed in the center of the stained area and mixed with the stain by stirring with the edge of a second slide. When thorough mixing has occurred, the stained blood is smeared across the remainder of the slide. It is allowed to dry and then is counterstained with Wright's stain. The number of reticulocytes per 1000 red blood cells should be enumerated to obtain an accurate count. The result is expressed as a percentage.

Relationship Between the Red Cell Count Hemoglobin and Volume of Packed Red Cells in Absolute Measurements Morphologically the anemias may be classified according to the size of the red cell normocytic microcytic and macrocytic. A hemoglobin concentration within normal limits is called normochromic below normal hypochromic. Hyperchromic anemias are nonexistent. The increase in hemoglobin in the macrocyte may run parallel with the size of the macrocyte but does not exceed it. Since the macrocyte is thicker than the normocyte the central pallor is less marked in the microcyte which appears dark when stained.¹

The indices calculated from the red cell count hemoglobin concentration and volume of packed red cells offer important clinical clues to treatment.

Color index The color index a widely used measurement is obtained by dividing the hemoglobin value in grams per 100 ml by the red cell count both values being designated as a percentage of normal. It defines the hemoglobin content of the individual cells as compared with the content of a normal cell. A color index of approximately 1 either is normal or connotes a normocytic normochromic anemia; an index of below 0.8 characterizes a hypochromic anemia; an index above 1 denotes macrocytosis which is usually associated with hypochromia.

Although the color index is in general a valid expression of the relationship between hemoglobin saturation and cell size exceptions occur. In infancy and early childhood especially the color index may be unreliable because of the normal fluctuations in hemoglobin and red cell counts which accompany growth.

$$\text{Color index} = \frac{\text{Hemoglobin (\% of normal [14.5 gm per 100 ml = 100\%])}}{\text{Red cells \% (5 million per cubic millimeter = 100\%)}}$$

More precise and instructive information regarding cell size and hemoglobin concentration in the younger age groups is obtained by measuring the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC).

Price Jones curve The Price Jones curve is a graphic method of recording the diameters of red blood cells of various sizes from the stained blood smear.⁷ In the majority of normal subjects the peak diameters are approximately 6.69 to 7.7 microns which are taken as the upper and lower limits of normal.¹⁰ The average mean diameter is given as 7.2 microns. The sizes of the red cells are noted on the abscissa and the number of the cells (frequency) in each size group on the ordinate. The results are plotted in frequency curves. Figures or curves above or below the two peaks indicate macrocytosis and microcytosis respectively. Anisocytosis is indicated by spreading of the base of the curve and flattening of the peak. The effects of treatment on microcytosis in patients with pernicious anemia are particularly well demonstrated in the Price Jones curve. Since measurement of the red cell diameters is a laborious procedure the simpler measurement of the mean corpuscular volume is frequently substituted for it.

Red cell indices The designation of the anemia according to size of the red cell and hemoglobin content serves to suggest specific blood disorders and their therapy.

MEAN CORPUSCULAR VOLUME (MCV) This represents the mean or average volume of a single red cell. The result is expressed in cubic microns.

$$\frac{\text{Hematocrit per cent} \times 10}{\text{Red blood cell count millions per cubic millimeter}}$$

Example

Red blood cell count	5 million
Hematocrit	45%
MCV	$\frac{45 \times 10}{5} = 90 \text{ cubic microns}$

Normal range MCV = 80 to 94 cubic microns

A MCV of more than 94 cubic microns indicate macrocytes 80 to 94 cubic microns normocytes less than 80 cubic microns microcytes

MEAN CORPUSCULAR HEMOGLOBIN (MCH) Mean corpuscular hemoglobin represents the average quantity (weight) of hemoglobin per individual red cell. Results are expressed in micromicrograms

$$\frac{\text{Hemoglobin grams per 100 ml} \times 10}{\text{Red blood cell count millions per cubic millimeter}}$$

Normal range MCH = 27 to 32 micromicrograms

A MCH with normal hemoglobin content is called normochromic above normal hyperchromic below normal hypochromic

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) This represents the average concentration of hemoglobin in the individual red cell as calculated from the amount of hemoglobin per 100 ml of cells rather than of whole blood. The result is expressed as percentage

$$\frac{\text{Hemoglobin grams per 100 ml} \times 100}{\text{Hematocrit per cent}}$$

Normal range = 32 to 38 per cent

Red cells with MCHC of less than 32 per cent are hypochromic. At approximately 34 per cent hemoglobin the normal erythrocyte contains a maximal number of hemoglobin molecules. Hyperchromia in reality describes an increased intensity of staining of the red cells. A cell with increased thickness as is observed in patients with hereditary spherocytosis gives the impression of hyperchromia (thickness above normal value of 2 millimicrons). MCH and MCHC measure the weight and concentration of hemoglobin respectively in the average red cell.

The absolute values of the mean corpuscular hemoglobin concentration and the mean cell volume offer practical guides for diagnosis and therapy. The combination of microcytosis (MCV below 80 cubic microns) and hypochromia (MCHC of less than 32 per cent) is indicative of iron deficiency. Characteristic is a blood smear with a predominance of hypochromic microcytes and pronounced central pallor. Thalassemia minor which possesses similar red cell indices and red cell morphology as iron deficiency anemia is characterized by a familial and hereditary pattern and the presence of A hemoglobin. On the basis of the blood smear the two conditions are virtually inseparable. In patients with the less common macrocytic anemias with mean red cell volumes ranging above 94 cubic microns restoration to normal values can be expected with liver, vitamin B₁₂ and folic acid therapy. Macrocytes may be fully saturated with hemoglobin or the pigment may be diminished; in the latter case iron therapy is also indicated.

In patients with chronic infections and in those with systemic diseases such as nephritis the red cells may be microcytic or normocytic with little or no decrease in the content of hemoglobin. Patients with these infections and diseases fail to respond permanently to antianemia therapy unless the primary cause is treated. Transfusions are symptomatically required for patients with these conditions as well as for those with the normochromic and normocytic anemias re-

sulting from bone marrow depression. These include aplastic and hypoplastic anemias and leukemia in which marrow cell displacement occurs. A normocytic normochromic anemia in a child for which no definite hematologic basis can be discovered necessitates comprehensive survey for an underlying pathologic condition.

Bone Marrow Examination. Aspiration of the bone marrow constitutes a useful laboratory aid in the diagnosis of blood disorders. The accessibility of the marrow, its responses to stimuli producing depression or hyperplasia, its availability for repeated examinations, and the comparative ease of identifying the cellular elements account for frequency with which aspiration is performed.⁸ Disturbances of each of the principal blood elements are frequently reflected earlier or are more conspicuous in the bone marrow than in the peripheral blood. In patients with leukemia, for instance, the bone marrow may be extensively infiltrated with leukoblastic cells, whereas they appear in the peripheral blood in such scant numbers as to be overlooked. In patients with hypoplastic aplastic anemias and in those with the hemolytic anemias, bone marrow studies permit quantitative estimation of the cells types involved. Bone marrow examination serves as a guide to therapy with transfusion agents and especially with the chemotherapeutic compounds in leukemia.

NORMAL VALUES. The figures in Table 6 represent the approximate range and average values of the cellular elements which one may expect to find in samples of the bone marrow obtained from normal infants and children. Certain modifications must be made in view of the changing pattern of the percentages of the component cells. The granulocytic series predominate in the first week or two of life. By one month the lymphocytes increase in number and often extend beyond the 25 per cent level noted in Table 6 to reach levels of 50 per cent during the first year. The presence of a lymphocytosis of this magnitude need not give rise to unnecessary concern, provided the cells are morphologically normal. Between 4 and 8 years of age the granulocytes equal the lymphocytes numerically and eventually predominate, reaching adult values at about 12 years of age.

Table 6 Normal Values of Cellular Elements in Bone Marrow in Older Infants and Children

	Range (Per Cent)	Average (Per Cent)
Myeloblasts	1 to 5	2
Myelocytes (including promyelocytes)	10 to 25	20
Nonsegmented polymorphonuclear cells (including metamyelocytes)	15 to 30	20
Segmented polymorphonuclear cells	5 to 30	25
Lymphocytes	5 to 25	13
Nucleated red cell (principally normoblasts)	15 to 30	20
Megakaryocytes	10 to 35 per cu mm	
Total nucleated cell count	100 000 to 200 000 per cu mm	

DIAGNOSTIC FEATURES IN THE BLOOD DISORDERS The individual cellular constituents in the bone marrow are in such close proximity to each other that it is difficult for one element to be affected independently of the others. This total stimulation is best observed in patients with the hemolytic anemias and most regularly in those with acute hemolytic anemia. In the latter regeneration of the red blood cells is associated with an increase in the number of granulocytes and platelets. The reverse occurs in patients with aplastic anemia in whom values for the three types of blood cells are depressed simultaneously. However, other factors must operate in addition to anatomic proximity since involvement of a single type of cell may occur. This is illustrated in persons with pure red cell anemia in whom the production of red blood cells is inhibited without equal depression of the other cellular elements. Similarly, selective hyperplasia occurs in persons with iron-deficiency anemia and in those with chronic loss of blood in whom the normoblasts and their immediate precursors alone are primarily increased.

In patients with the hemolytic anemias (namely erythroblastosis fetalis, hereditary spherocytosis, sickle cell anemia, thalassemia major, and acquired hemolytic anemia) the bone marrow is hyperplastic and there is an increased

Table 7 Diagnostic Features of the Bone Marrow in the Common Anemias of Infancy and Childhood

Type of Anemia	Diagnostic Features
Hemolytic group (erythroblastosis fetalis, hereditary spherocytosis, sickle cell anemia, thalassemia major, acquired hemolytic anemia)	Hyperplastic marrow with increase of nucleated red cells; chiefly normoblasts; granulopoiesis may also be active; occasionally during crises marrow aplastic instead of hyperplastic.
Iron deficiency anemia	Hyperplastic marrow with increase in normoblasts; granulopoiesis unchanged.
Hemorrhagic anemia	Hyperplastic marrow with increase in nucleated red cells; chiefly normoblasts; granulopoiesis active in acute hemorrhage and unchanged in slow and chronic loss of blood.
Megaloblastic anemia of infancy	Pattern similar to pernicious anemia in relapse; megaloblastic type of erythropoiesis and changes in granulocytes resembling pernicious anemia.
Aplastic anemia	Usually decreased cellularity to acellular; sharp reduction in myeloid elements; megakaryocytes and normoblasts; increase in lymphocytes.
Hypoplastic anemia	Hypoplastic marrow with pronounced depression affecting normoblasts mainly.
Aregenerative (pure red cell) anemia	Normal marrow except for absence of nucleated red cells.

proliferation of normoblasts and to a lesser extent of erythroblasts. Granulopoiesis may be active especially in persons with acquired hemolytic anemia. In patients with iron deficiency anemia and in those with chronic hemorrhagic anemia the bone marrow is similarly hyperplastic and values for normoblasts are greatly elevated but granulopoiesis is normal. In patients with acute hemorrhage however, the activity of the myeloid elements is also increased. The increase of the nucleated red cells in patients with these disease processes in excess of 50 per cent of all the nucleated elements in the marrow constitutes an important diagnostic feature (Table 7).

Aplastic anemia is associated with profound anemia, leukopenia, neutropenia and thrombocytopenia. The bone marrow shows a progressive decrease in cellularity and there is a sharp reduction in the myeloid elements, megakaryocytes and nucleated red cells with a relative increase in normal lymphocytes. Occasionally acute lymphoblastic leukemia in the leukopenic stage may simulate aplastic anemia and diagnosis is difficult without histologic examination of the bone marrow. Bone marrow aspiration in patients with leukemia will reveal a cellular bone marrow in which the normal nucleated elements are replaced by primitive cells of the lymphoid or myeloid series. With the progressive reduction in the number of platelets hemorrhages occur in patients with aplastic anemia which may simulate thrombocytopenic purpura. The bone marrow in persons with idiopathic thrombocytopenic purpura reveals a hyperplasia of immature megakaryocytes. In secondary thrombocytopenic purpura occurring in patients with leukemia and aplastic anemia megakaryocytes are reduced in number or are entirely absent in the counting chamber and the bone marrow smears.

INVASION OF THE BONE MARROW BY FOREIGN CELLS. Abnormal elements characterizing a specific pathologic condition occasionally appear in the bone marrow. In younger age groups these consist most commonly of Gaucher and Niemann-Pick cells, the abnormal histiocytes of Letterer-Siwe disease (nonlipid reticuloendotheliosis) and metastatic neoplastic cells such as are found in patients with neuroblastoma and lymphosarcoma.

Characteristic tumor cells are found with such great frequency in patients with metastatic neuroblastoma that bone marrow examination is essential when this disease is suspected. In one series of cases the abnormal cells were found in fifteen of thirty-one infants and children. Their presence is not dependent upon the existence of radiovisible bone lesions. In patients with this condition ball-like masses or clusters of large immature cells form syncytial masses or pseudorosettes with a mosaic pattern.⁵ The individual cells are large with nuclei possessing an immature chromatin pattern which stain deep blue. The cytoplasm is scant faintly basophilic and without granules.³

TECHNIQUE OF BONE MARROW ASPIRATION. The sternal manubrium is rarely the optimal site for bone marrow aspiration in children. Other sites offer the advantages of being less dangerous, less painful and less emotionally traumatic to the patient. Such sites are the lower thoracic or lumbar vertebral spinous processes, anterior iliac crest, posterior iliac crest and tibia. In children older than 18 months to 2 years the spinous processes are most useful. In younger children or infants selection of the iliac crest may expedite the procedure. It is probably wise

to reserve use of the tibia for exceptional circumstances since fractures have resulted from such punctures at this site

Skin preparation should include the use of pHisoHex as well as benzalkonium. Anesthesia is obtained by the use of 1 per cent procaine solution. Following the intradermal injection of approximately 0.2 ml of the procaine solution a short time interval is necessary to allow adequate anesthetic effect to occur. If this step is observed it is possible to ensure an almost painless procedure. The

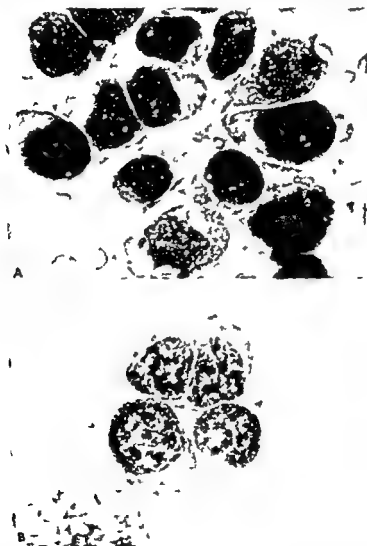


Fig 4 Bone marrow smears in neuroblastoma. A Note typical pseudorosette formation around fibrillar network. B Same formation as shown in A showing finer detail of individual cells in circular grouping ($\times 1200$) (A Courtesy Dr Ralph L. Engle, Jr., New York, N. Y.)

subcutaneous tissue is next infiltrated with proper care being taken to avoid touching the periosteum. Another short interval of time will allow further specific injection of the periosteal layer without discomfort to the patient.

An 18 gauge $3/4$ or 1 inch needle is used in the majority of children although a smaller gauge may be employed for very young infants. Once the tip of the needle has been inserted through the periosteum the needle is inserted with added pressure until it is firmly fixed within the bone. A sense of "give" is only occasionally felt as the needle enters the marrow cavity. The point of insertion should be in the midpoint of the spinous process or just 1 cm below the lip of the ileum. The angle of insertion should be perpendicular to the spinous process or to the flat surface of the ileum. At all times the needle should be guarded at the skin's surface by the fingers of the operator's left hand to prevent it from slipping off the bony prominence while pressure is being exerted.

The stylette is then removed and a 10 ml syringe is attached to the needle. The actual aspiration of marrow contents is usually painful. It may be wise at this point to warn the patient that if pain occurs it will probably signify the end of the procedure. To minimize admixture with blood only 0.1 to 0.2 ml of marrow is withdrawn into the syringe. Release the suction in the syringe. This maneuver is necessary to avoid spreading the aspirate along the walls of the syringe when the needle is being removed from the bone. Remove the needle still attached to the syringe from the puncture site and expel the aspirate onto a glass slide. At this time speed is essential to avoid early coagulation and loss of cells especially megakaryocytes.

A total nucleated cell count is made by diluting the fluid marrow in 3 per cent acetic acid as for a peripheral white cell count. Cell types which may be particularly notable in the counting chamber include megakaryocytes and the large cells of Gaucher's and Niemann Pick diseases.

Smears are made on glass slides which have been washed in 85 per cent alcohol. Wright's stain is satisfactory for routine use.

Contraindications to bone marrow aspiration are hemophilia and related coagulation disorders. However the bone marrow can be safely aspirated in thrombocytopenic purpura. Bone marrow fragments can be isolated from the aspirated sample and can be fixed and sectioned or be examined unstained for their fat content and hemosiderin granules. In patients with iron deficiency hemosiderin is absent or present in only small amounts. It is thought by many that smears from these spicules offer a more accurate estimate of the cellular content and arrangement of the bone marrow than smears from the total aspirate. The differentiation between hyperplasia and hypoplasia of the bone marrow can be estimated by the total number of nucleated cells in the counting chamber or by inspection of the smear.

Myeloid Erythroid Ratio The myeloid erythroid ratio provides an index of depression or hyperactivity of the granulocytic elements as compared with nucleated red cells. In the newborn infant the M:E ratio rises from 2:1 on the first day of life to about 10:1 or 12:1 in the second week, indicative of granulocytic hyperplasia and a decline of erythrocyte production. Beyond infancy the M:E ratio is 2.5 to 3.5:1.¹¹

Röntgenographic Examination The greater value of the roentgenogram in the diagnosis of blood dyscrasias in earlier life than in later childhood and adult life can be related to the developmental features of the bone marrow. The fact that all the bones are filled with red marrow is advantageous to the infant and young child in whom the demand for erythropoiesis is so active. Fat appears in substantial amounts in the long bones at about 7 years of age. Only with the appearance of nonfunctioning yellow marrow in the older child and its extension in the young adult is a potential reservoir available for the emergency formation of blood. In the absence of yellow marrow the infant or young child faced with the need for excessive blood formation reactivates extramedullary fetal sites. In addition the marrow hypertrophies and expands by absorption and atrophy of the bony trabeculae and the cortex. These changes are observed in the roentgenograms and are of value in diagnosis. More detailed discussions of the changes observed by roentgenography are described in connection with the specific blood disorders in this book and in the book by Caffey.¹

Following is a list of the bone and joint manifestations of the blood disorders of infancy and childhood.

Disorders affecting blood cells and bone marrow

- 1 Red cells
 - A Aplastic anemia (Fanconi syndrome)
 - B Hereditary hemolytic anemias
 - (1) Cooley's (thalassemia) anemia
 - (2) Hereditary spherocytosis
 - (3) Sickle cell anemia
- 2 White cell
 - A Leukemia
- 3 Bone marrow
 - A Marble bone disease
 - B Myelofibrosis

Coagulation disorders

- 1 Classical hemophilia (antihemophilic globulin deficiency)
- 2 Christmas disease (plasma thromboplastin component deficiency)

Hereditary hemolytic anemias (severe Cooley's anemia, sickle cell anemia, hereditary spherocytosis)

Common features

- 1 Blood: increased hemolysis and blood production
- 2 Bone changes due to compensatory marrow hypertrophy
- 3 X-ray changes in skeleton including skull and vertebrae
- 4 Bone changes most marked in severe Cooley's anemia and least in spherocytic anemia

A Cooley's anemia

- 1 Skeletal changes confined to severe cases
 - (A) Dilatation of marrow cavities
 - (B) Pressure atrophy of epiphyses and cortices
 - (1) Concave surfaces of metacarpals and long bones change to rectangular shape
 - (2) Osteoporosis changing to sclerosis with age
 - (3) Maturation and growth retarded
 - (4) Pathologic fractures

- 2 Skull changes
 - (A) Widening of diploic space hair on end appearance (also reported in patients with chronic iron deficiency anemia)
 - (B) Swelling of zygomas
- 3 Vertebral changes reticulated occasionally compressed

II Sickle cell anemia

- 1 Marrow hyperplasia less marked than in Cooley's anemia
- 2 Skull changes as in Cooley's anemia
- 3 Long bones in children slight to no changes in adults fibrosis and thickening of cortex
- 4 Aseptic necrosis usually in femoral head less common in humeral head
 - (A) Destructive and productive changes due to capillary thromboses and infarction
 - (B) Occurs in sickle cell anemia sickle-hemoglobin C disease and sickle cell-thalassemia disease
- 5 Tendency toward osteomyelitis in salmonella infection
- 6 Vertebrae—compression deformity

C Hereditary spherocytosis

- 1 Skeletal and cranial changes as in Cooley's anemia but infrequent and less marked cranial changes more prominent than skeletal

Fanconi syndrome

- 1 Aplastic anemia in combination with congenital defects
 - (A) Affecting heart skin mentality and genital development
 - (B) Skeletal—variable absence of thumb calcareal bones radius syndactylum

Osteopetrosis (marble bone disease Albers Schonberg disease)

- 1 Cause—failure to resorb calcified cartilaginous matrix
- 2 Increased thickness and density of the cortical and spongy portions of the entire osseous system bones brittle
- 3 X-ray—individual bones and skull opaque heavy and lacking in finer structure
- 4 Tendency toward fractures slipping of epiphyses

Leukemia

- 1 Skeletal changes from encroachment upon functioning marrow by leukoblastic cells observed in metacarpals long bones and pelvis
- Destruction of spongiosa erosion of cortex lifting of periosteum
Osteoporosis cystic rarefaction moth eaten appearance of long bones
- 3 Transverse zones of diminished density in metaphyses of long bones

Hemophilia

- 1 Skeletal lesions due to
 - A Bleeding directly into the bones
 - (1) Shafts and epiphyses
 - (a) Rounded defects in spongiosa
 - (b) Cystic areas of rarefaction
 - (2) Subchondral
 - (a) Marginal bony defects at juxta articular borders—flattening deformity of proximal epiphyses of femur (like coxa plana)
 - (3) Subperiosteal hematomas
 - (a) Atrophy of underlying cortex
 - B Bleeding into joint spaces
 - (1) Retained blood and clots
 - (a) Inflammation deformities and ankyloses destruction of articular cartilage thickened synovial membrane connective tissue invasion into joints
 - (b) Generalized rarefaction of epiphyses and shafts

(2) Regional hyperemia and recurrent hemarthroses result in hypertrophy of adjacent epiphyses

C Hemarthroses—marked in both classical hemophilia (antihemophilic globulin deficiency) and in Christmas disease (plasma thromboplastin component deficiency)

Hereditary Factors as an Aid in Diagnosis The genetic aspects of disease are manifested in a variety of hematologic disorders notably erythroblastosis fetalis the hereditary hemoglobinopathies the group of primaquine sensitive hemolytic anemias hereditary spherocytosis and elliptocytosis and the coagulation defects. Specific genetic implications are discussed in connection with each entity elsewhere in the book. The discovery of hereditary factors depends upon the application of selected laboratory tests made not only on the affected patient but also on his asymptomatic relatives for the signs of the trait. Tests in common use include examination of the blood smear for morphologic abnormalities fragility tests serologic techniques starch and paper electrophoresis exposure of red cells to specific chemical agents to determine their tendency toward hemolysis coagulation studies and a comprehensive investigation of the family for incidence of the disease. An analysis of the data provides insight into the genetic control of these disorders extending to their qualitative representation.

Thalassemia sickle cell anemia and hereditary spherocytosis may be cited as examples of familial diseases in which the hereditary trait may be recognized by suitable blood studies. Their hereditary nature is reflected in the relatively high incidence in members of the same family. The diagnosis of these diseases in a child with an obscure anemia of moderate severity often can be made earlier in its course and useless therapy avoided by detection of the trait in the parents and siblings. In the patient with thalassemia for instance the most important element in the diagnosis of the milder form with the simple means immediately available to the practitioner is the discovery of qualitatively similar alterations in the blood of other members of the same family. These persons are asymptomatic and have either mild anemia or no anemia. Regardless of the hemoglobin level their blood shows hypochromic macrocytes stippled erythrocytes polycythemia increased resistance of the red cells to hemolysis in hypotonic solutions of sodium chloride and less frequently target or oval cell. The evidence is conclusive that in every family with a child having thalassemia major requiring periodic transfusions both parents reveal evidence of the disease. When this bilateral inheritance of the gene for thalassemia is nonexistent a search is made by electrophoresis and sickle cell preparations for the presence of another abnormal hemoglobin in the seemingly unaffected parent.

Diagnostic Features of the Blood Smear Of all laboratory procedures the most important for its diagnostic value yet the simplest is the examination of the blood smear. Although corroborative evidence from auxiliary sources may be required to establish a final diagnosis the stained blood film constitutes a visual representation of the effect on morphology of the factors involved in the pathogenesis of a specific anemia. In describing the changes in the red blood cells it should be pointed out that except for sickle cells spherocytes and leptocytes (the large pale extremely thin ridged erythrocytes with irregularly distributed hemoglobin observed in the blood of patients with thalassemia major) there are no specific red cells that are indicative of a particular disorder. Even the small deeply stained spherocytes which characterize congenital spherocytic anemia may also be observed in greater or lesser degree in patients with other conditions associated with hemolysis such as acquired hemolytic anemia erythroblastosis due to sensitization by the A B agglutinogens and leukemia. Target cells oval cells

hypochromic macrocytes and basophilic stippling and hypochromic microcytes appear in varying percentage in certain stages of many anemias and therefore cannot be regarded as distinguishing features of a single disease. They are of

*Table 8 Diagnostic Features of the Blood Smear in the Anemias of Early Infancy**

Type of Anemia	Diagnostic Features
Erythroblastosis fetalis	Hyperchromic macrocytes nucleated red blood cells
Hemorrhagic anemia	Acute blood loss normochromic and normocytic red blood cells Chronic blood loss hypochromic microcytes
Iron deficiency anemia	Hypochromic microcytes marked central pallor of red blood cells poikilocytes
Anemia of prematurity	Early—normocytic and normochromic red blood cells later—hypochromic microcytes
Megaloblastic anemia of infancy	Unusually large macrocytes microcytes occasional nucleated red blood cells abnormal neutrophils

From Smith C H. Anemias in Infancy and Childhood. Diagnostic and Therapeutic Considerations. Bull New York Acad Med 30 153 1954

*Table 9 Diagnostic Features of the Blood Smear in the Anemias of Later Infancy and Childhood**

Type of Anemia	Diagnostic Features
Anemia of infection	Normocytic and normochromic / occasionally microcytic and hypochromic red blood cells with iron deficiency hypochromic microcytes predominate
Aplastic hypoplastic and aregenerative (pure red cell) anemia	Normochromic and normocytic red blood cells
Acquired hemolytic anemia	Moderate spherocytosis marked polychromasia reticulocytosis
Congenital non spherocytic hemolytic anemia (may appear at birth)	Normochromic and normocytic reticulocytosis occasionally macrocytosis anisocytosis and poikilocytosis
Hereditary spherocytosis (may appear at birth)	Spherocytes polychromasia reticulocytosis
Sickle cell anemia	Sickle cells target cells microcytes occasional nucleated red cells
Thalassemia major (Cooley's anemia)	Very large thin hypochromic macrocytes nucleated red blood cell marked poikilocytosis anisocytosis
Thalassemia minor	Hypochromic macrocytes microcytes basophilic stippling target and oval cells

*From Smith C H. Anemias in Infancy and Childhood. Diagnostic and Therapeutic Considerations. Bull New York Acad Med 30 155 1954

diagnostic value only when they are considered in conjunction with pertinent information from other sources. The morphologic changes of the red cells in the most common anemias of infancy and childhood are summarized in Tables 8 and 9. Each of these disorders is described in greater detail elsewhere in the book.

Principles of Treatment The information gathered thus far has provided a sufficiently firm foundation with respect to pathogenesis to warrant a consideration of therapy. In Table 10 are listed the available agents in current use designed to correct an underlying deficiency or to ameliorate the disorder by accessory means when direct remedies are not available. The constant emphasis on the use of specific rather than indiscriminate mixtures of antianemic substances¹¹ applies with especial force to the pediatric age group. Iron salts given orally, preferably in the ferrous forms without the aid of supplementary minerals or vitamins still constitute the most effective treatment of iron deficiency anemia. Treatment with iron salts in patients with other conditions may not only be futile but also potentially harmful. The anemias for which transfusions are indicated consist of two groups: those in which this measure constitutes a temporary expedient until spontaneous recovery sets in and those in which, in the absence of specific therapy, the need for restoration of optimum blood levels is continuous or urgent. The indications for splenectomy will be discussed in Chapter 25.

Allergic Implications of Blood Disorders The clinical manifestations of many of the blood disorders are wholly or partially dependent upon immunologic reactions. The type of response resulting from the combination of an antigen with an antibody has its counterpart in the immunologic mechanisms underlying many of the blood disorders. Many of these are now classified under the heading

Table 10 Therapy of the Common Anemias of Infancy and Childhood

Agent	Type of Anemia
Iron	Iron deficiency anemia; late anemia of prematurity; chronic blood loss
Folic acid—ascorbic acid(?) vitamin B ₁₂ (?)	Megaloblastic anemia of infancy
Transfusion	Erythroblastosis; nonhemolytic anemia of newborn infant; anemia due to hemorrhage and infection; aplastic; hypoplastic and pure red cell (chronic congenital or degenerative) anemias
Splenectomy	Spleroctytic anemia; acquired hemolytic anemia in selected cases; thalassemia; major sickle cell anemia; aplastic anemia; hypoplastic anemia; and pure red cell anemia
ACTH and steroids (including testosterone)	Acquired hemolytic anemia; aplastic; hypoplastic and pure red cell anemia (?) crises of sickle cell anemia (?)

of immunohematology and include the hemolytic anemias, granulocytopenia and thrombocytopenia. The pathogenesis is based to some extent on antibodies to the respective formed blood elements, blood vessel hypersensitivity, incompatibility between blood elements of fetus and mother, transfusion reactions and sensitivity induced by bacterial, chemical and physical agents.²⁴ Hypersensitivity to drugs may result in hypoplastic or aplastic anemia in contrast to primaquine sensitive hemolytic anemias which are based on an inherited intrinsic defect of the red cells.

It is of interest to consider the extraordinary phenomenon by which antibodies which are capable of reacting with the patient's own red cells are elaborated. A similar situation occurs in those patients with thrombocytopenic purpura and agranulocytosis in whom antibodies are directed against the patient's platelets and polymorphonuclear cells. These disorders call attention to the remarkable tolerance developed from early fetal life by which the body in health avoids forming antibodies against its own tissues. Such dire circumstances in which a living organism becomes capable of producing antibodies against its own body constituents was termed "horror autotoxicus" by Ehrlich.²⁵ The fundamental feature of autoimmune acquired hemolytic anemia and other disorders characterized by autoimmune antibodies to blood cell elements is the loss of this normally acquired tolerance with the development of antibodies to one's own cellular antigens.

CAUSATIVE FACTORS

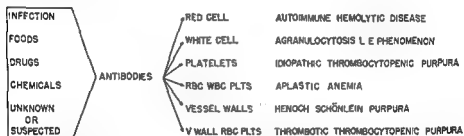


Fig. 5. Immunoallergic concept of blood disorders. (From Smith, C. H. *Allergic Implications of Blood Disorders in Infancy and Childhood*, California Med. 86:366, 1957.)

Once the effects of the hypersensitive state are set in motion during the course of a blood disorder, therapeutic measures to slow their progress are often futile. Because it is not always possible to identify the potentially allergic child in whom these circumstances will occur, it is extremely important to weigh the advantages of the use of a drug before it is administered, especially when its side effects have not yet been thoroughly investigated.

Following is a list of blood disorders in infancy and childhood, many of whose clinical and laboratory features lend themselves to an interpretation on an immunoallergic basis (also see Fig. 5).

Disorders affecting blood cell and vascular elements

1. Red cells
 - A. Acquired hemolytic anemia
 - (1) Toxic products, drugs, favism

- (2) Antibodies against red cells
 - (a) Erythroblastosis fetalis
 - (b) Blood transfusion reactions
 - (c) Associated with autoantibodies primary autoimmune hemolytic anemia secondary leukemia Gaucher's disease
 - (d) Pure red cell (chronic congenital aregenerative) anemia
- 2 White blood cells
 - A Agranulocytosis
 - (1) Anticonvulsants
 - (2) Antimicrobial agents
 - (3) Antithyroid drugs
 - (4) Sulfonamides
 - B Neonatal agranulocytosis
- 3 Platelets
 - A Thrombocytopenic purpura
 - (1) Idiopathic
 - (2) Congenital
 - (3) Drug induced quinine quinidine sulfonamides arsenicals
 - (4) Eczema with purpura and otitis media
- 4 Red cells white cells and platelets
 - A Hypoplastic and aplastic anemia
 - (1) Idiopathic
 - (2) Drugs chemical infection
- 5 Blood vessels
 - A Allergic purpura (Henoch Schonlein syndrome)
 - B Idiopathic pulmonary hemosiderosis
 - C Polyarteritis nodosa and other collagen diseases
 - D Thrombotic thrombocytopenic purpura (vascular vessel wall in combination with platelets and red cells)

Infections

- 1 Infectious mononucleosis
- 2 Susceptibility following splenectomy

Autoerythrocytic Sensitization The symptoms in this condition are easy bruising with swelling edema, and painful ecchymoses due to autosensitivity of patients to their own blood. Severe trauma with bruising characterize the history. A stromal factor of red cells may be the responsible agent for the clinical manifestations and for the positive skin tests. Extremely painful reactions follow intradermal testing with red cells. This syndrome corresponds to the type of auto sensitization noted in patients with lupus erythematosus some forms of acquired hemolytic anemia and thrombocytopenic purpura.⁴

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Iron-Deficiency Anemia

Iron deficiency anemia represents the most common nutritional deficiency in children and is especially prevalent in infancy. The unusual susceptibility of the infant is due to a variety of interrelated factors inherent in this age period which affect iron metabolism.

Etiology Iron deficiency anemia occurs most commonly between the ages of 6 and 24 months and reflects either an inadequate supply or an excessive demand for iron. These factors are interdependent and overlap. In the first six months the infant is dependent upon iron reserves acquired during fetal life. With the depletion of these stores the infant requires dietary iron to meet the demands of growth. The period before and after 6 months of age cannot be sharply circumscribed because of the individual variability of iron stores and the extent of growth. The major etiologic factors in iron deficiency in infancy and childhood are as follows:

- 1 *Inadequate supply of iron*
 - A Inadequate iron stores at birth
 - (1) Premature twin or multiple births
 - (2) Severe iron deficiency in the mother
 - (3) Fetal blood loss at or before delivery
 - (a) Fetal hemorrhage into maternal circulation
 - (b) Retroplacental bleeding
 - B Inadequate intake—deficient dietary iron
- 2 *Impaired absorption*
 - A Chronic diarrhea
 - B Celiac disease
 - C Gastrointestinal abnormalities
- 3 *Excessive demands for iron*
 - A Blood loss during infancy
 - (1) Acute or chronic hemorrhage
 - (2) Parasitic infection such as hookworm
 - B Failure to meet increased demands for growth
 - (1) In the premature infant and in other rapidly growing infants
 - (2) During adolescence

In a series of 272 infants whose blood was found to contain less than 9 gm of hemoglobin per 100 ml Woodruff⁸ found that the most common cause of

hypochromic anemia was prematurity or a birth weight of less than 3 kg. The incidence of severe hypochromic anemia in infants weighing more than 4 kg was extremely low. A first child was less likely to become anemic than were later siblings, single children were less likely than twins, and girls less likely than boys.⁸ Guest and Brown⁶ also found a significantly lower incidence of iron deficiency anemia in first born infants than later born infants.

Prenatal factors play an important role in influencing the iron content of the fetus. These include the length of gestation, maternal hemoglobin concentration, and less frequently, occult or frank hemorrhage from the fetal surface of the placenta at or before delivery. Abnormal placental circulation in multiple births, especially in single ovum twins, may result in the transfusion of one twin by another.³⁶ Such transfusions may explain the relatively frequent occurrence of hypochromic anemia in only one twin regardless of similar growth rates and diets.⁸

The only source of iron for the fetus is derived transplacentally from the maternal organism. To provide for increased needs incident to the rapid growth of the fetus in the last trimester of pregnancy, iron absorption in the mother is accelerated.⁴⁸

The average adult has about 3 to 5 gm of iron in his body, of which 2 to 3 gm are found in the hemoglobin, 1 to 1.5 gm in the body stores as ferritin and hemosiderin, and the rest in myoglobin, respiratory enzymes, and the plasma. The cytochromes are iron-containing porphyrin compounds which function as respiratory enzymes in the cell. In contrast, at birth the infant's supply is approximately 300 mg, provided by the mother; after birth iron for the increasing needs of the growing body is derived from the diet.

Although the iron transfer to the fetus is negligible during the first two trimesters of pregnancy, it rises in the third trimester to 4 mg daily so that the total amount accumulated by the fetus at term approximates 300 mg. Widdowson and Spray⁴⁹ found an average of 273 mg of iron in six infants weighing more than 3,000 gm. They found that the iron concentration during fetal life was relatively constant with an average value of 75 mg per kilogram of body weight. Of this, the liver and spleen contained one eighth of the total (34 mg). The bulk of iron available to the infant at birth for eventual hemoglobin formation is that contained in the circulating hemoglobin. Josephs⁴⁷ estimated approximately 30 to 35 mg in the liver. The amount of the nonhemoglobin iron fraction (myohemoglobin, cytochromes, and other respiratory enzymes) at birth has been variously estimated at 4 mg per kilogram,⁴⁷ 6 mg per kilogram,⁴⁸ and 7.5 per kilogram. The total hemoglobin mass may be computed by multiplying the blood volume of 85 ml per kilogram (variation 80 to 90 ml per kilogram) by the hemoglobin concentration per 100 ml of blood. Since each gram of hemoglobin contains 3.4 mg of iron, the total hemoglobin value is multiplied by this figure to convert it to total body iron. With these values Sturgeon calculated⁴⁷ in a hypothetical infant weighing 4 kg that 232 mg of iron is present in circulating hemoglobin, 51 mg in the liver and spleen, and 16 mg as myoglobin and other parenchymal iron. In an infant weighing 3 kg at birth the figures accordingly would be 106 mg from circulating hemoglobin, 11 mg in stores, and 12 mg in nonhemoglobin iron.

Premature twin and multiple births reduce the quantity of iron in the storage depots. These factors also tend to reduce the total amount of circulating hemoglobin and therefore the content of iron required for later hemoglobin synthesis. Less frequently, reduced stores result from hemorrhage due to loss of fetal blood into maternal circulation,⁴ from placental previa, from abruptio placentae, and

from injury to the cord during delivery. Accidental bleeding from the cord following delivery reduces the initial iron stores in relation to the extent of hemorrhage. Early or late clamping of the cord is another important factor contributing to the iron endowment of the infant. Manual stripping of the cord can add as much as 75 ml of whole blood or 40 mg of iron to the storage depots.⁶⁷ It has been emphasized⁶ that the technique of delayed ligation of the cord does not result in a significant increase in red cell volume unless the cord is initially stripped or milked.

Full term infants born to nonanemic mothers receive sufficient iron during fetal life to meet their needs for at least the first three to four months of infancy.^{81, 8} With few exceptions infants born to mothers who have suffered iron depletion during pregnancy show normal hemoglobin concentrations at birth. The influence of an inadequate iron endowment does not become manifest until later in the first year. In markedly anemic mothers administration of iron before delivery of their babies prevents the development of anemia in the newborn infant.^{60, 8} Unless the anemia in the mother is marked (below 9 gm per cent) iron deficiency usually does not occur in the infant.⁸¹ Despite these facts maternal iron depletion as indicated by severe anemia is not regarded as a common predisposing factor of iron deficiency in infants in the United States at the present time.¹ Administration of iron supplements to normal mothers overcomes the mild physiologic anemia of pregnancy but exerts little if any influence on the offspring's iron endowment at birth or iron nutrition in infancy.^{68, 69}

Iron deficiency anemia in the full term infant which is noted at approximately 6 months of age cannot be evaluated properly unless important hematologic events in the preceding period are considered. These two periods are not sharply circumscribed but one merges with the other since both are affected by forces within and without the hematopoietic system. Of basic importance is the fact that the iron depots at birth possessing variable amounts of this mineral (less in the premature and more in the full term infant) are substantially increased by progressive accumulation of iron in the first months of life.⁴⁰ This major iron supplement is derived from the degradation of circulating hemoglobin mass with which the infant is born. Regardless of the amount the initial iron tissue stores at birth cannot be underestimated for their value in hemoglobin synthesis.

Relation of Physiologic Anemia of the Newborn Infant to Iron Deficiency Anemia. Following an initial week or ten days of stable or mildly fluctuating blood levels the hemoglobin and red cells drop reaching a minimum at approximately 7 weeks of age in the premature infant and at 2 to 3 months in the full term infant. This phase designated as physiologic anemia of the newborn infant is highly important in the iron economy of the infant since the iron liberated by red cell destruction is stored for later use in rebuilding hemoglobin. During this period the red cell count falls proportionately to hemoglobin so that a normochromic normocytic anemia develops. It should be emphasized that the anemia at this stage is due to a depression of erythropoiesis and hemoglobin synthesis during which red cells are destroyed at normal or at best slightly increased rates.⁴

hypochromic anemia was prematurity or a birth weight of less than 3 kg. The incidence of severe hypochromic anemia in infants weighing more than 4 kg was extremely low. A first child was less likely to become anemic than were later siblings, single children were less likely than twins, and girls less likely than boys.⁸ Guest and Brown⁹ also found a significantly lower incidence of iron deficiency anemia in first born infants than later born infants.

Prenatal factors play an important role in influencing the iron content of the fetus. These include the length of gestation, maternal hemoglobin concentration, and less frequently, occult or frank hemorrhage from the fetal surface of the placenta at or before delivery. Abnormal placental circulation in multiple births, especially in single ovum twins, may result in the transfusion of one twin by another.¹⁰ Such transfusions may explain the relatively frequent occurrence of hypochromic anemia in only one twin regardless of similar growth rates and diets.^{5,6}

The only source of iron for the fetus is derived transplacentally from the maternal organism. To provide for increased needs incident to the rapid growth of the fetus in the last trimester of pregnancy, iron absorption in the mother is accelerated.^{7,11}

The average adult has about 3 to 5 gm of iron in his body, of which 2 to 3 gm are found in the hemoglobin, 1 to 1.5 gm in the body stores as ferritin and hemosiderin, and the rest in myoglobin, respiratory enzymes, and the plasma. The cytochromes are iron-containing porphyrin compounds which function as respiratory enzymes in the cell. In contrast, at birth the infant's supply is approximately 300 mg, provided by the mother, after birth, iron for the increasing needs of the growing body is derived from the diet.

Although the iron transfer to the fetus is negligible during the first two trimesters of pregnancy, it rises in the third trimester to 4 mg daily, so that the total amount accumulated by the fetus at term approximates 300 mg. Widdowson and Spray¹² found an average of 273 mg of iron in six infants weighing more than 3,000 gm. They found that the iron concentration during fetal life was relatively constant with an average value of 75 mg per kilogram of body weight. Of this, the liver and spleen contained one eighth of the total (34 mg). The bulk of iron available to the infant at birth for eventual hemoglobin formation is that contained in the circulating hemoglobin. Josephs¹³ estimated approximately 30 to 35 mg in the liver. The amount of the nonhemoglobin iron fraction (myohemoglobin, cytochromes and other respiratory enzymes) at birth has been variously estimated at 4 mg per kilogram,¹⁴ 6 mg per kilogram,¹⁵ and 7.5 per kilogram.¹⁶ The total hemoglobin mass may be computed by multiplying the blood volume of 85 ml per kilogram (variation 80 to 90 ml per kilogram) by the hemoglobin concentration per 100 ml of blood. Since each gram of hemoglobin contains 3.4 mg of iron, the total hemoglobin value is multiplied by this figure to convert it to total body iron. With these values, Sturgeon¹⁷ calculated¹⁸ in a hypothetical infant weighing 4 kg, that 232 mg of iron is present in circulating hemoglobin, 51 mg in the liver and spleen, and 16 mg in myoglobin and other parenchymal iron. In an infant weighing 3 kg at birth, the figures accordingly would be 106 mg from circulating hemoglobin, 11 mg in stores, and 12 mg in nonhemoglobin iron.

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typical systolic murmur is frequently heard but the heart is not enlarged. The changes in the epithelial structures characterizing iron deficiency in adults such as atrophic glossitis, dysphagia and spoon shaped or concave nails (koilonychia) do not occur in infants and are only rarely observed in older children. Because of gradual development of the anemia signs of cardiac dysfunction are usually not encountered even in infants with severe grades of hemoglobin reduction.

Laboratory Data. Important laboratory information is obtained from a study of the peripheral blood, bone marrow and plasma of patients with iron deficiency anemia.

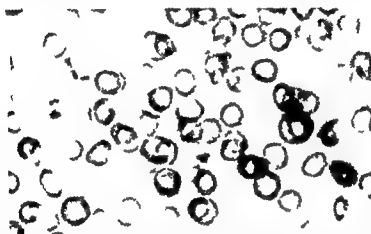


Fig. 6. Blood smear of an infant with iron-deficiency anemia. Note predominance of hypochromic microcytes fairly uniform in size and shape whose outstanding feature is marked deficiency in hemoglobin indicated by central pallor ($\times 1350$). (From Smith C. J. *Treatment of the Anemias of Infancy and Childhood*. J. Pediat. 32:21, 1918.)

Blood. The anemia of iron deficiency is characteristically hypochromic and microcytic in contrast to the normochromic normocytic type of physiologic anemia in the postnatal period. Since iron deficiency primarily affects hemoglobin synthesis and red cell formation to a lesser degree the red cell count may be normal, near normal or moderately reduced. The hematocrit levels are lower than normal. The decreased hemoglobin content of the individual erythrocyte is the specific abnormality present in iron deficiency anemia. The hemoglobin is reduced out of proportion to the red cell count. The red cell count varies usually ranging from 3 to 5 million with a hemoglobin level from 3 to 9 gm. per 100 ml. The following criteria of hypochromic anemia may be designated: hemoglobin concentrations below 9 gm. per 100 ml., a mean corpuscular hemoglobin (MCH) less than 27 micrograms, a mean corpuscular hemoglobin concentration (MCHC) of less than 30 per cent, and a mean corpuscular volume (MCV) of less than 78 cubic microns.¹

The blood smear shows microcytes, poikilocytes, elliptical and elongated pencil forms and target cells. Many of the red cells are diminutive in size. A small

The diminished hematopoiesis is explained by Gardner and associates¹⁸ as an adjustment to the changeover from the placenta to the lung as a source of oxygen. To maintain a normal postnatal oxyhemoglobin content of the blood there is no need for elevated hemoglobin levels so that erythropoiesis and hemoglobin synthesis are retarded until such a time as the hemoglobin level drops from elevated blood levels at birth to approximately 11 to 12 gm per 100 ml. Any decrease below this point induces increased hematopoietic activity whereas elevations above this level will tend to decrease marrow activity. When the designated concentrations are reached in the full term infant at 2 to 3 months of age marrow erythroid activity is resumed¹⁹ as reflected by reticulocytosis and the gradual rise in the circulating hemoglobin mass. A fundamental principle in the kinetics of iron metabolism is that once iron is introduced into the body it is used again and again. The amount excreted at any one time is small and is assumed to be less than 1 mg daily.

At approximately 16 weeks of age in the full term infant the total amount of circulating hemoglobin is restored to the initial birth values. A humoral erythropoietic stimulating factor, erythropoietin,¹ may also be involved in these fluctuations, functioning when anemia develops and being inactive at the high hemoglobin concentrations at birth.

With the resumption of active erythropoiesis, reticulocytes appear and the circulating mass expands. In both full term and premature infants the amount of hemoglobin synthesized will be limited by the iron which has accumulated in the stores during the postnatal drop in hemoglobin. This in turn depends principally upon hemoglobin concentration and hemoglobin mass available at birth and supplemented by a variable component of pre-existing iron in the liver and spleen. Increase in weight and the blood volume obscures the improvement in the hemoglobin concentration during the period of recovery.

The supply of iron becomes strained with rapid growth so that the infant is exposed to an anemia based on the exhaustion of storage iron. Following the period of reutilization of iron and hemoglobin synthesis and increase in total hemoglobin mass, the supply of this mineral becomes depleted unless the stores are replenished by exogenous iron from an adequate diet. These demands are met in the average full term infant. The failure to add foods containing significant quantities of iron leads to iron deficiency anemia, especially if the demands are excessive. Iron deficiency anemia accordingly is observed in clinical practice in infants who are growing rapidly and are fed almost exclusively on diets of milk and cereals with low iron content and present feeding difficulties.

Clinical Features. Infection which plays a dominant role as an etiologic factor in anemia in infancy and childhood frequently highlights an underlying state of iron deficiency. However, pallor, irritability, increased listlessness and gastrointestinal disturbances usually direct attention to severe grades of this disorder. A history of repeated upper respiratory difficulties is not uncommon.

In no condition is the sheetlike or waxy pallor so striking as in advanced iron deficiency anemia. Except for pallor of the skin and mucous membranes and occasionally a moderately enlarged spleen, the patients present no significant physical abnormalities. As in patients with other severe anemias, a soft blowing

microcytic picture and other diagnostic aids are necessary. The recent discovery of increased levels of A hemoglobin in the mild type of the disease (thalassemia heterozygotes) has proved to be of confirmatory value.¹⁸

Confusion with the severe homozygous type of Cooley's anemia may occur only in the infant in the first year of life. The large pale extremely thin erythrocytes with irregularly distributed hemoglobin and scattered normoblasts interspersed among microcytes in the blood smear are in contrast with the more uniform microcytes of iron deficiency anemia. Occasional hypochromic microcytes are however also found in the latter. The significant splenomegaly and the presence of the trait in both parents and siblings further differentiates Cooley's anemia from iron deficiency anemia.

Changes of the skull identical with the hair standing on end appearance observed on the roentgenogram of patients with the chronic hemolytic anemias have been described in patients with chronic iron deficiency anemia especially in premature infants and twins.¹⁹⁻²² These changes are associated with erythroid hyperplasia in the bone marrow. In contrast to changes observed in patients with Cooley's anemia these changes are confined to the skull. The roentgenograms of the long bones fail to reveal any abnormality.

Treatment. It has been established clinically and in the laboratory that treatment with simple iron salts is effective in correcting iron deficiency anemia. Nevertheless successful management requires a thorough investigation of basic etiologic factors such as faulty diet, blood loss, and malabsorption due to chronic diarrhea or a structural gastrointestinal defect such as polyps or Meckel's diverticulum. Most commonly the history will reveal a dependence on foods notably poor in iron content such as milk, cereals, and other carbohydrate foods. Often this situation will have developed unwittingly from a failure of parents to understand the need for a well balanced diet and its particular importance in the rapidly growing infant. Each of these factors can be eliminated by diagnostic and therapeutic procedures and the diet can be adjusted. Restriction of milk to one pint a day, the introduction of meat, vegetables, and fruit, and supplementation by an iron preparation usually will suffice to correct the anemia.

Of importance in all infants, the full term and especially the premature, is the insistence upon serial hemoglobin determinations from the second month and at appropriate intervals thereafter for the detection and control of the anemia. The necessity for this measurement stems from the variability in the hemoglobin mass at birth from which iron is derived for later hemoglobin synthesis and the individual needs for exogenous iron due to growth of tissues and expanding blood volume.

To achieve maximum improvement in hemoglobin concentration Sturgeon²³ has suggested that the recommended daily allowance of 6 mg. of food iron through the first year and 10 mg. from 1 to 3 years of age be increased to intakes of 6 to 9 mg. daily at 3 months of age, to 8 to 12 mg. by 6 months, and to 10 to 15 mg. at 12 months. One egg yolk supplies approximately 1 mg. of iron and per each the best source of iron among the fruits provide 0.3 mg. per tablespoon.²⁴

number of normal sized red cells and microcytes are usually present often with a normal hemoglobin content. The characteristic appearance of the blood smear is the presence of a majority of small red cells with marked central pallor with fine and poorly staining rims of hemoglobin. Occasional stippled red cells and normoblasts may be present. Reticulocytes are normal or reduced. The fragility of the red cells may be normal or may show a moderate resistance to hemolysis in hypotonic solution of sodium chloride. The platelet count is normal. The leukocytes are usually normal or slightly reduced.

Bone Marrow The bone marrow shows erythroid hyperplasia with a pre dominance of polychromatophilic normoblasts often smaller than normal. The cytoplasm may be diminished to the extent that it forms a small rim around the nucleus. Granulopoiesis is normal. Megakaryocytes are normal in number and in appearance. Contributory evidence of diminished iron stores may be directly determined by bone marrow examination which reveals a decrease of iron granules in the normoblasts (sideroblasts)^{10,34} and an almost complete absence of stainable iron or hemosiderin in marrow aspiration. This depletion of hemosiderin is contrasted with the increased quantities noted in infection and in hemolytic anemias.^{31,64}

Plasma The serum iron is reduced varying from 10 to 60 micrograms per 100 ml and averaging about 30 micrograms per 100 ml (normal approximately 120 micrograms). The latent iron binding capacity of the serum is increased to approximately 450 micrograms and above (normal 250 micrograms per 100 ml). The saturation of serum iron (serum iron divided by serum iron plus latent iron binding capacity) is reduced and averages 6 per cent⁶¹ (normal 32 per cent). A greatly reduced serum iron and a markedly elevated latent iron binding capacity have not been found in any other condition than iron deficiency and are therefore of diagnostic value. With treatment the serum iron is increased the latent iron binding capacity undergoes a moderate contraction and serum iron saturation increases. In addition to hypoferrremia and reduced saturation of total serum iron other chemical manifestations of iron deficiency include increased blood copper levels (hypercupremia) and increased free erythrocyte protoporphyrin.⁶⁶

Of the many diagnostic features delineating the hematologic picture of an anemia due to a primary deficiency of iron the simplest is the presence of microcytic hypochromic red cells which dominate the stained blood smear.

Diagnosis The characteristic small pale red blood cells of iron deficiency anemia in the stained smear, a history of an iron deficient diet and the marked pallor of the patient are usually sufficient to make the diagnosis in the infant over 6 months of age. The stool examination for unrecognized chronic blood loss is essential.

Iron deficiency anemia and milder forms of thalassemia are often indistinguishable. The red cells in both conditions show moderate stippling and target and oval forms but the larger number of hypochromic macrocytes in the blood smear and the familial hereditary pattern are important diagnostic features of thalassemia minor. The blood smear of patients with mild thalassemia and of those with iron deficiency may both show however a more or less uniform hypochromic

salts or because it shares with other foods of increased phosphorus content a basic difficulty in absorbing iron.⁴ Phytates contained in cereals also make non-ionized precipitates with iron. Despite these findings iron salts in therapeutic doses added to milk, however, have produced satisfactory hemoglobin responses in our experience and in that of others.¹⁰ Furthermore the iron in iron supplemented cereals now in common use in infant feeding is also well absorbed.

Oral administration of iron usually results in the stool becoming a deep black color due to the increased content of iron sulfides. The absence of this change may serve as a clue to the irregular administration of the iron.⁸ Ingestion of liquid iron preparations may produce a black staining of the teeth. Brushing the teeth after each administration is of value in reducing this untoward effect although the staining is only temporary.

For older children tablets of ferrous sulfate or ferrous gluconate are preferable to the concentrated liquid preparations used with infants. Gastric irritation, nausea, vomiting, and abdominal pain are less likely to occur if the tablets are taken with meals. Iron tablets are best taken three times daily at mealtimes. Tablets of ferrous sulfate (0.2 gm. 3 grains) or ferrous gluconate (0.3 gm. 5 grains) given three times daily provide a daily total of 100 to 200 mg. of elemental iron.

In the infant as in the older child full therapeutic doses of iron should be given for at least six to eight weeks after the hemoglobin has been restored to normal levels. If oral therapy is withdrawn too soon the iron stores will remain un replenished¹¹ and anemia will eventually recur.

Iron dextran for intramuscular use. Parenteral iron therapy is indicated when patients fail to respond to adequate oral dosage because of gastrointestinal irritation, gastrointestinal pathology, or more rarely refractoriness to oral therapy because of failure to absorb iron. An iron dextran mixture for intramuscular use is now available (Imferon[®]) which has proved a valuable adjunct to therapy. This preparation is well tolerated and provides 50 mg. of elemental iron in each milliliter. The total amount of iron needed to raise the hemoglobin level to normal and replenish stores is calculated as follows:

$$\frac{\text{Normal hemoglobin} - \text{Initial hemoglobin}}{100} \times \text{Blood volume (milliliters)} \times 3.4 \times 1.5$$

1. Normal hemoglobin = 11 to 12 gm. in infants, 12 to 13.5 gm. in children, 14 to 15 gm. at puberty.
2. Blood volume = 80 ml. per kilogram or 40 ml. per pound of body weight.
3. 3.4 converts grams of hemoglobin into milligrams of iron.
4. Factor 1.5 provides extra iron to replace depleted tissue stores.

Example. Infant weighing 10 kg. with 5 gm. of hemoglobin per 100 ml.

Blood volume = 80 ml. per kilogram \times 10 kg. = 800 ml.

Hemoglobin deficit = 12 gm. (approximately normal for age) - 5 gm. = 7 gm. per 100 ml.

Total hemoglobin deficit = 7 gm. $\times \frac{800}{100}$ = 56 gm.

Iron to restore hemoglobin to normal = 56 gm. \times 3.4 mg. = 190 mg.

Iron to replenish stores = 50 per cent of 190 mg. = 95 mg.

Total dose of iron = 190 + 95 = 285 mg. of iron.

At present Imferon has been temporarily withdrawn from the market.

Iron rich foods include the following commercially prepared dry ready to serve infants cereals (0.92 mg of iron per tablespoon) green and yellow vegetables (0.05 mg per table spoon in spinach) green beans (0.28 mg per tablespoon) and liver (0.56 mg per tablespoon)

Josephs gives the iron content of milk as follows. As it comes from the cow it contains about 0.4 to 0.5 mg of iron per liter. Commercial pasteurization increases the iron to 0.7 to 1.0 mg per liter. Processed milk whether powdered or condensed may contain 1 to 2 mg per liter. Breast milk contains 0.7 to 1.0 mg per liter.

Iron Therapy. Treatment with a soluble iron salt preferably ferrous iron corrects the deficiency more promptly than the ingestion of foods rich in iron although both should be given simultaneously. Ferrous iron permits significantly higher hemoglobin values in well children than does ferric iron of the same dosage.⁴⁶ Ingested iron is absorbed in the ferrous form largely from the duodenum. Ferrous sulfate is the preparation of choice in most infants because the metallic iron content is adequate and it is efficiently absorbed, inexpensive and easily administered. Normal infants will absorb an average of 10 to 20 per cent of orally administered ferrous sulfate in solution and iron deficient children absorb 5 to 10 per cent more.⁶

Normal children absorb an average of about 10 per cent of the naturally occurring iron in milk, eggs, chicken liver and iron supplements added to commercially prepared infants cereals.⁴⁸ Iron deficient children absorb 2 to 3 times as much food iron as do normal children.⁴⁸ In normal adult subjects on the contrary approximately 5 to 15 per cent absorption takes place from ferrous salt given in 100 mg amounts.⁴⁹ With uncomplicated iron deficiency anemia the absorption is increased to between 25 and 70 per cent. With food iron the percentage of absorption is 1 to 10 per cent which is increased to 20 to 30 per cent in the iron deficient subjects. Reducing substances especially ascorbic acid enhanced the absorption of food iron. With nearly 5 to 10 per cent of iron in food assimilated by normal adults the daily retention on a diet containing 12 to 15 mg is approximately equivalent to the amount of iron lost from the body each day.

DOSAGE AND MODE OF ADMINISTRATION. Since the percentage of metallic iron content is a fraction of the entire compound iron should be prescribed with this fact in mind. Such preparations are now available and are suitably designated according to iron content.

For infants ferrous sulfate is available in concentrated solutions which can be given in drop dosage in which each drop contains a measured amount of elemental iron. The total dose of elemental iron recommended for an infant during the period of iron deficiency (6 to 24 months) ranges from 60 to 90 mg. Ideal treatment consists of the administration of such a soluble iron preparation in divided dosage preferably between meals.

There are a large variety of iron salts ferrous and ferric alike available in solution form that produce a satisfactory hemoglobin response. The ferric solutions require a somewhat higher dosage. The pediatrician should have knowledge of several preparations with respect to details of administration and content of elemental iron since it is occasionally necessary to interchange them because of gastrointestinal irritation or intolerance produced by one or another preparation.

Milk usually has been regarded at least theoretically as being an unfavorable vehicle for iron either because it combines with phosphates to form insoluble

Cobalt has been advocated in the treatment of anemia because of the known development of polycythemia when it is fed to experimental animals. Plasma from animals injected with cobaltous chloride rapidly develops a high titer of erythropoietin.²⁷ Cobalt is an essential element in vitamin B₁₂ (cyanocobalamin). Despite these considerations and reported beneficial results in the treatment of anemia associated with renal disease,²⁸ cobalt has been found lacking as a therapeutic agent in patients with iron-deficiency anemia, hypoplastic anemia, and other instances of bone marrow failure. Of even greater concern is its possible toxicity in some patients producing a goitrogenic effect in young infant,²⁹ hence the danger of recommending cobalt alone or cobalt with iron for clinical use.

Copper is normally found in the blood in both the plasma and the red corpuscles. Serum copper increases in pregnancy, infection, and iron deficiency. In adult males the concentration of copper in the serum is 105 micrograms per 100 ml with a standard deviation of ± 16 .³⁰ In normal infants beyond 8 months of age the serum copper values are elevated. The range from 140 to 200 micrograms per 100 ml is evidence of iron depletion during infancy.³¹

Experimental studies have shown that copper plays a role in the absorption and utilization of iron.³² With serious depletion of copper not only is iron absorption impaired but also erythropoiesis is decreased and the defective erythrocytes that are produced have a shortened survival.³³

In spite of the striking evidences of anemia in copper-deficient experimental animals the usefulness of copper in the therapy of anemia in man has still to be demonstrated.³⁴ Copper is known to exist as an impurity in the therapeutic iron salts. It is of interest that processed baby foods such as strained beef liver and cereals and, to a lesser extent, fruits and vegetables possess the highest level of copper. These foods are certainly sufficient to ensure the infant's daily requirement of copper of 0.05 mg per kilogram of body weight per day.³⁵ The major portion of copper in the plasma is found in the A₂ globulin ceruloplasmin.

Transfusions With the availability of suitable iron preparations for oral and parenteral administration, transfusions are left for the infant with a hemoglobin level of about 4 gm per 100 ml or less who is debilitated from an infection, especially when signs of cardiac dysfunction are present. With such severe grades of anemia, hospitalization is safer than ambulatory treatment.

Anemia of the Premature Infant The tendency of the premature infant to develop subnormal hemoglobin levels as compared with the full term infant results from the impact of identical forces which are exaggerated in the premature infant. The differences between the anemia of prematurity and the physiologic anemia of the full term infant are quantitative. The premature infant is subject to two phases of anemia: one early and the other late. The early anemia is present at the end of the neonatal decline; the late anemia dates from that period in the first year when the iron stores are exhausted. In both the premature and full term infant the initial period is one of declining hemoglobin levels due to the decreased rate of hemoglobin synthesis. Both subsequently undergo a stage of hemoglobin regeneration from reutilization of iron released from the destroyed red cells. The late anemia in the premature infant is one of iron deficiency when exogenous iron is not in adequate supply to meet the demands of growth. Characteristic in the premature infant are the more accelerated drop and the lower levels of hemoglobin which are obtained in the postnatal period as compared with the steadier and more prolonged drop in the hemoglobin level in the full term infant. In the premature infant, erythropoietic activity begins therefore at 5 to 7 weeks of age, which is earlier by a month or more than in the full term infant.

The relatively lower initial hemoglobin mass at birth of the premature infant

The total dosage according to age has also been estimated by Wallerstein and Howell⁴ as follows: 100 mg. for infants under 6 months of age, 200 mg. under 12 months of age, 300 mg. from 12 to 24 months of age, and 400 mg. over 24 months of age. Injections are given daily or when this is impractical at weekly intervals. In small infants the initial dosage is 50 mg. (1 ml.) With daily injections newly formed red cells well filled with hemoglobin are recognizable in blood smears within forty-eight hours after therapy. About the fifth day of treatment reticulocyte counts reach maximum values which are probably initiated after twenty-four hours of therapy. In patients with severe anemia the hemoglobin level reaches 11 gm. per 100 ml. after three weeks and higher in patients with moderate anemia. The magnitude of the hemoglobin response varies therefore with the degree of anemia. If hemoglobin values do not rise at least 2 gm. in three weeks other possible causes of anemia should be considered and more iron should not be administered.

Injections into the upper outer quadrant of the gluteus muscle are given through skin which has been disinfected laterally prior to the injection to prevent superficial stinging. In rare cases local or generalized reactions, angioneurotic edema and recurrent arthritis occur.⁴

RESPONSE TO ORAL IRON THERAPY. The recovery is reflected by a reticulocyte response that reaches its maximum on the fifth to the tenth day after the institution of iron therapy. The magnitude of the response is related to the degree of anemia. Following the reticulocyte rise the hemoglobin level, volume of packed cells (hematocrit) and red cell count increase. Following the peak of the reticulocyte rise the hemoglobin rises at an average of 0.17 gm. to 0.25 gm. per 100 ml. per day. A substantial hemoglobin rise should be observed at approximately three weeks after beginning iron therapy. The failure to achieve a level of at least 11 gm. per 100 ml. in this period with adequate iron therapy indicates that the diagnosis of anemia on a purely nutritional basis is to be questioned and suggests the continuation of an infectious process and underlying renal abnormality, a continued blood loss, or impaired absorption.

It is unwise to increase the dosage in refractory cases for it is theoretically possible to induce hemosiderosis by indiscriminate administration of excessive amounts of parenteral iron. Iron given parenterally is stored and a maximum daily excretion of only 1 mg. compels selectivity of patients and control of treatment by this route.

ADJUVANTS TO IRON THERAPY. Adjuvants to iron are unnecessary in the treatment of iron deficiency anemia. It is unnecessary to add copper, molybdenum, or cobalt supplements to obtain an adequate response. Nor are vitamin B₁₂ and folic acid required in treatment of iron deficiency anemia. Since iron is absorbed in the ferrous form, the reduction of ferric salts to the bivalent form depends upon reducing mechanisms present in the small intestine. Moore and his associates⁴⁵ demonstrated that the ingestion of vitamin C with ferric salts resulted in an increase in serum iron, probably by its reducing action. On the other hand, in a study of children of school age Schulze and Morgan, employing soluble ferric pyrophosphate and small supplements of copper, found that the addition of ascorbic acid was unnecessary for the synthesis of hemoglobin.

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The relatively lower initial hemoglobin mass at birth of the premature infant

the decreased synthesis of hemoglobin in the first weeks of life and the very rapid growth and expansion of blood volume constitute the fundamental factors involved in the pathogenesis of the anemia. A decreased life span in the erythrocytes of the premature infant has also been cited as a cause of the more exaggerated drop of the hemoglobin in the initial phase,¹⁴ but more extensive data are needed with regard to this factor.

Management. The greater tendency of iron deficiency in the premature infant prompts a consideration of the most favorable time for iron supplementation. Most premature infants exhaust iron stores (derived from the hemoglobin mass at birth) by the fourth month of life.¹⁵ Seip and Hiltvorsen¹⁶ found little or no storable iron in the bone marrow of the premature infant immediately after birth and a marked increase between 4 and 5 weeks of age. Iron stores decreased until finally no hemosiderosis could be demonstrated at 8 to 12 weeks of age. In the full term infant, on the contrary, the bone marrow did not become hemosiderin free until 16 to 20 weeks of age. It is questionable whether it is necessary to administer iron prophylactically to premature infants during the postnatal period of declining hemoglobin and red cell values when the bone marrow is nonreactive and when iron from degraded hemoglobin is being conserved. Available evidence indicates that iron administered soon after birth fails to retard the postnatal drop in hemoglobin but does reveal its effectiveness in infants with higher values from the third to the fourth month. Essentially the same results are observed when iron is given by injection. When iron dextran (Imferon) was given intramuscularly over a period of two to four days during the second or third week of life, a significant increase in hemoglobin was not observed until the infant was 3 months of age. The iron administered by this method accelerates the recovery of the early anemia and prevents the late (iron deficiency) anemia.¹⁴

A dosage of 30 to 45 mg. of elemental iron given from two months should protect the majority of premature infants from iron deficiency anemia and allow for iron storage. In instances in which iron deficiency already exists the dosage is increased to 60 to 90 mg. daily. The iron is given in divided doses according to the principles outlined for iron deficiency anemia in the full term infant. In most premature infants iron should be continued for several months after the hemoglobin concentration has returned to normal to ensure adequate stores. Periodic hemoglobin determinations are required to assure the maintenance of normal blood values. Prophylaxis by means of parenteral iron (iron dextran) may be advisable before discharge from the hospital when it is anticipated that oral administration will be unreliable. The dosage in this case is 100 mg. of parenteral iron.

The management outlined for the premature infant also applies in cases of multiple births. In instances in which the hemoglobin level drops to 7 gm. per 100 ml. or less between 3 and 6 weeks of age, transfusions are required especially when the infant is listless, eats poorly, and is not gaining weight. It should be mentioned however that besides the known complications of transfusions there is the possibility that repeated administration of blood may suppress inherent hematopoiesis at a time when the bone marrow may have become responsive.

Iron Transport—Serum Iron and Iron Binding Capacity Recent studies dealing with the metabolism of iron with respect to the phases of absorption, transport and storage have provided another diagnostic tool in characterizing an anemia based on iron deficiency. Iron entering the plasma is derived from heme pigments from body stores and from gastrointestinal absorption. It is transported in the plasma in combination with a specific protein, a beta₂ globulin variously designated as metal combining globulin, siderophilin or transferrin, whose movement is responsible for the redistribution of iron in the body. This protein carries two iron atoms in the ferric state. In the process of iron turnover the transferrin delivers the metal to the liver, spleen or bone marrow, either for storage in a labile iron pool or for hemoglobin synthesis and erythropoiesis. Iron is stored both as ferritin and as hemosiderin, which are equally available for use in erythropoiesis. Ferritin is soluble and not visible in the tissues, whereas hemosiderin (a polymer of ferritin) is insoluble and is easily discerned as golden granules when unstained or as blue granules after staining with potassium ferrocyanide. The major portion of iron leaving the plasma is normally directed toward the bone marrow where it is used for hemoglobin synthesis.³

Normally transferrin is about one third saturated; the bound fraction representing the segment linked to iron as serum iron. The unsaturated or latent capacity of this protein to combine with additional iron is subject to measurement. Serum iron is defined as the acid soluble iron in the serum; the latent (unsaturated) iron binding capacity is defined as that amount of additional iron capable of being bound by the unsaturated protein in serum. The total iron binding capacity represents the sum of these two components. The percentage of saturation represents the percentage of iron binding protein which is saturated with iron and is calculated by dividing the serum iron by the total iron binding capacity. The total iron binding capacity constitutes in effect an indirect estimate of the circulating iron binding protein.

Granick²⁴ has suggested that iron is absorbed by the mucosal cells of the duodenum in the ferrous form and stored there as ferritin. Upon entering the mucosal cells the iron apparently is oxidized to the ferric form and as ferric hydroxide polymerizes and is attached in micelles or clusters to the colorless iron free protein, apoferritin. It is thus iron containing form which is known as ferritin. A mucosal block against excessive absorption is said to occur when the available apoferritin is fully saturated with iron. In the presence of an anemia it has been postulated that the relative tissue anemia creates a greater reducing tendency in the intestinal mucosal cells. The conversion of the ferric iron present as a ferritin to circulating ferrous iron is thereby enhanced. This ferrous iron then rapidly attaches itself to the iron binding protein, transferrin, where it is converted to the ferric form. An additional amount of apoferritin is thus made available and iron is once again absorbed through the intestinal mucosa.

According to this concept, control of iron absorption is mediated through the intestinal mucosa which can refuse or accept iron depending upon the state of the stores. Evidence has been increasingly available that in patients with refractory anemia, pernicious anemia in relapse, hemolytic anemia and hemochromatosis the mucosal block does not prevent iron from being absorbed in spite of adequate body iron stores.²⁵ Such data throw doubt upon ferritin and the mucosal cell as sole regulators of iron absorption and demonstrate that the mucosal block to iron absorption is only relatively complete.

Except for local factors within the intestine (phosphates phytates) the state of iron stores and the rate of erythropoiesis are major factors in the control of iron absorption.⁴ Absorption is increased when the erythroid marrow is hyperactive (hemorrhagic hemolytic anemia) or when iron stores are depleted (iron deficiency). Contrariwise absorption of iron is reduced when iron stores are increased and when erythropoiesis is depressed as in the plethora produced by transfusion.

Iron Binding Capacity of Plasma in Various Clinical Conditions The level of serum iron reflects the balance between the iron absorbed the iron utilized for hemoglobin synthesis the iron released by red cell destruction and the size of the storage depots. More simply stated at any particular time the serum iron concentration represents a precise equilibrium between the iron entering and leaving the circulation.

In persons with iron deficiency anemia the depleted stores are represented by a greatly reduced serum iron a markedly expanded iron binding capacity and a very low percentage of saturation. An increase in the total iron binding capacity may be interpreted as an added facility for transporting this metal and is therefore an indication for iron therapy to replenish stores. In patients with acute and chronic infection the impairment of hemoglobin synthesis is accompanied by a disturbance in erythropoiesis and a diversion of iron to tissue stores.^{51, 52} Both the serum iron and the latent iron binding capacity are significantly reduced but the percentage of saturation is not decreased to the extent noted in patients with iron deficiency anemia. In patients with disorders in which hemoglobin synthesis and marrow function are depressed as in those with untreated pernicious anemia hypoplastic anemia and aplastic anemia the serum iron is greatly elevated and the latent iron binding capacity is reduced and often absent when multiple transfusions have been given. In persons with conditions of iron excess such as the hemolytic anemias transfusion hemosiderosis and hemochromatosis the body iron is markedly increased as a result of the preponderance of red cell destruction over formation and in part from increased iron absorption. When the serum iron is markedly elevated the latent iron binding capacity is absent so that the saturation is 100 per cent. As shown in Fig 7 and Table 11 the examination of the individual patterns materially advances the differentiation of the various anemias and provides an insight into the separate phases of iron metabolism.

Fig 7 is based on the following average values for serum iron and latent iron binding capacity respectively in micrograms per 100 ml for normal children 122 and 246 micrograms iron deficiency 27 and 472 micrograms Cooley's anemia and the hemolytic anemias 203 and 0 micrograms. The column for chronic infection represents a typical case in a child in this instance with findings of 37 and 200 micrograms per 100 ml respectively and corresponds to the average adult figures given by Cartwright and Wintrobe⁵³ of 30 and 165 micrograms per 100 ml.

The values for serum iron and iron binding capacity in normal infants and children at various ages are given in Table 12.⁵¹ The iron values noted in the period from 5 to 8 days of age represent a persistence of the findings in cord

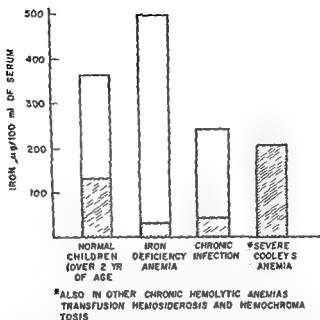


Fig. 7 Schematic representation in specific anemias. Shaded areas represent serum iron and clear areas latent iron binding capacity. The total iron binding capacity is the sum of these two fractions. The high serum iron and absent latent iron binding capacity in patients with the hemolytic anemias are contrasted with the low serum iron and greatly expanded latent iron binding capacity in those with iron-deficiency anemia.

Table 11 Serum Iron and Iron Binding Capacity in Various Clinical Conditions

Decreased	Increased
Serum Iron	
Iron deficiency	Hemolytic diseases
Acute and chronic infection	Hemosiderosis (transfusion)
Pregnancy	Hemochromatosis
Debilitating diseases (uremia, cancer, etc.)	Pernicious anemia (untreated)
	Cirrhosis
	Acute hepatitis
Latent Iron Binding Capacity	
Infection	Iron deficiency
Hemolytic disease	Pregnancy
Debilitating diseases	
Cirrhosis	
Hepatitis	
Pernicious anemia (untreated)	
Hemochromatosis	Absent
Transfusion hemosiderosis	

blood—namely, a higher serum iron and lower latent and total iron binding capacities is compared to normal serum iron and higher latent and total iron binding capacities in the mother. These findings represent the combined effects of transplacental iron transfer to build up fetal stores and an increased demand for iron in the mother so that she becomes relatively iron deficient. At about

Table 12 Normal Values of Serum Iron and Iron Binding Capacity
(Values in Terms of Micrograms per 100 ml Serum)*

	Serum Iron	Latent Iron Binding Capacity	Total Iron Binding Capacity	Percentage Saturation
Adults	100	200	300	34
Infants				
5-8 days	148	114	262	65
1-2 yr	95	319	414	22
Children				
2-6 yr	116	249	365	38
6-12 yr	124	214	340	38

From Smith C H: Anemia in Infancy and Childhood. Diagnostic and Therapeutic Consideration. Bull New York Acad Med 30:155, 1954.

the end of the first month erythropoietic function is gradually resumed and the movement of stored iron in the direction of the bone marrow results in a low serum iron and high latent and total iron binding capacities most marked at 2 years of age. This pattern consistent with an iron deficiency state despite the absence of anemia indicates a continuous drain on storage depots.⁶⁷ As has been indicated overt anemia in which the serum iron shrinks further and the latent iron binding capacity expands occurs with rapid growth dietary deficiency and other causative factors. In general there is a direct correlation between the concentrations of serum iron and the state of iron storage.

Serum iron and iron binding determinations may be employed as useful guides in gauging the adequacy and completeness of therapy in patients with iron deficiency states. Iron administered orally or parenterally in patients with iron deficiency anemia is utilized quantitatively for hemoglobin synthesis. If iron is administered parenterally only in amounts calculated to restore blood values to normal or if oral therapy is terminated as soon as normal levels are reached the iron stores remain unreplenished. It is for this reason that iron therapy is continued for one or two months beyond the period when normal hemoglobin levels are achieved.

Hemosiderosis and Hemochromatosis Hemosiderosis refers to excessive iron shortage in various tissues of the body without associated tissue damage. Hemochromatosis is the term applied not only to a widespread increase in storage iron but also to resultant tissue damage.¹⁶ The clinical and pathologic changes associated with excessive dietary intake of iron among Bantu natives suffering

from malnutrition (cytosisiderosis) ⁶ and with the deposition of iron due to multiple blood transfusions (transfusion hemosiderosis) may not always be distinguishable from those of classical hemochromatosis (bronze diabetes pigment cirrhosis). The latter a disease predominantly of middle aged men is characterized by cirrhosis of the liver diabetes skin pigmentation hypogonadism and congestive heart failure. The underlying defect in this disease is a disturbance in the normal mechanism of the intestinal mucosa for preventing unnecessary iron absorption so that excessive quantities of iron are absorbed regardless of saturated body stores.⁷ During the initial phase of excessive iron absorption the plasma iron is elevated and the liver and reticuloendothelial tissues show an increased iron content. Eventually the liver becomes the chief site of iron storage.¹⁶

In patients with refractory anemias such as aplastic anemia aregenerative anemia and chronic hemolytic anemia iron stores are increased by virtue of multiple transfusions (250 mg in each 500 ml of blood) greater iron absorption and prolonged iron therapy.⁷ Nutritional siderosis of the African Bantu natives and siderosis resulting from excessive parenteral iron therapy fall into the same category so that the complete histopathologic changes similar to classical hemochromatosis eventually develop.¹

Whether or not hemosiderosis progresses to classical hemochromatosis is still being debated. Although the extensive deposition of iron in tissues is a significant characteristic of hemochromatosis this factor alone may not be the sole pathogenic factor in producing tissue changes.³ In patients with Cooley's anemia for instance chronic anemia and tissue hypoxia may constitute the essential factors in the conversion of hemosiderosis to hemochromatosis.^{14,30} Finch and Finch¹⁶ state that there are no unique features in idiopathic hemochromatosis by which it can be distinguished pathologically from the terminal stage of other iron storage diseases such as dietary or transfusion hemochromatosis.

Diagnostic Procedures Excessive body iron stores are indicated by an elevation of the plasma iron binding protein and excessive hemosiderin deposits in the bone marrow. Actual diagnosis is only made by the demonstration of pigmentary cirrhosis of the liver.

Treatment Mobilization of iron stores in idiopathic hemochromatosis has been successfully achieved by repeated phlebotomies.^{11,16,4} In children having huge iron stores with refractory anemia such as Cooley's anemia and pure red cell anemia the need for maintaining adequate hemoglobin levels precludes the use of this measure. The intravenous injection of chelating agents increases the urinary excretion of iron but this fix has been impractical in treatment of conditions in which massive iron overload exists.

Transient Dysproteinemia (Copper Deficiency in Infants) Several groups of infants have been described^{30,79,7} who show pallor edema irritability associated with varying grades of hypochromic microcytic anemia hypoproteinemia hypocupremia and hypoferremia in the first year of life. Hypocupremia in these infants is contrasted with the usual increase in serum copper in those with iron deficiency anemia. In one group these features were attributed to a primary abnormality in plasma protein marked by an increased rate of plasma protein degradation⁷³ without a compensatory rate of plasma protein synthesis. In other

groups patients were receiving cow's milk as their sole source of food intake³⁹. It is probable that two clinical states with differing causes operate in these cases⁴¹ one with a primary plasma protein deficiency and the other with a nutritional deficiency due to an exclusive milk diet. Improved diet, the corrective effect of copper sulfate and iron in various infants suggest that recovery occurs spontaneously and is unrelated to iron or copper therapy⁴¹.

Reviewing this syndrome Schubert and Labin⁴² observed that iron deficient infants cannot only be classified into those with and those without hypocupremia and hypoproteinemia but that intermediate or transitional forms exist. Hypoalbuminemia or hypocupremia and hypoalbuminemia occurred in infants with varying degrees of iron deficiency. The primary defect that they emphasized was one of iron deficiency and with gradual development of moderately severe anemia a protein deficient state develops which in turn leads to dysproteinemia and copper deficiency.

Hypoproteinemia has also been shown to result not only from excessive rates of protein destruction (hypercatabolic hypoproteinemia) or from a failure of protein synthesis but also from the loss of plasma protein into the gastrointestinal tract. This process termed exudative enteropathy⁴³ can be tested by the intravenous injection of ¹²⁵I labeled polyvinylpyrrolidone—a plasma protein substitute—which gaining access to the lumen of the gastrointestinal tract can be measured quantitatively by its radioactivity when excreted in the stool. ¹²⁵I labeled polyvinylpyrrolidone is excreted in the stools of normal subjects to the extent of 0 to 1.5 per cent of the dose and up to 20 per cent in those with exudative enteropathy. Lesions of the gastrointestinal tract may or may not exist. Hypoproteinemia, edema and iron deficiency anemia have been described in children with this syndrome. Anemia in these patients in spite of the absence of gastrointestinal bleeding is explained on the basis of nonhemoglobin iron lost in the stool^{71b}.

Acute Iron Intoxication The accidental ingestion of large doses of medicinal iron preparations usually ferrous sulfate tablets results in a fairly characteristic set of clinical features^{11, 12, 71} with a mortality of 50 per cent⁶. Early symptoms include vomiting, diarrhea, dehydration and the local effects due to the corrosive action of the metal on the stomach and other areas of the intestinal tract. Later evidences of severe and often irreversible cardiovascular collapse, shock and coma may be associated with the marked increases in plasma iron. Postmortem examination reveals dilatation of the right heart, pulmonary congestion, hemorrhagic and necrotizing gastritis, cloudy swelling and areas of hemorrhage in the lungs, brain, kidneys and liver.

Treatment of iron poisoning is largely symptomatic consisting of forced vomiting and prolonged and copious gastric lavage with sodium bicarbonate solution⁶. Shock is treated with blood, plasma and oxygen. BAL and chelating agents have also been employed. Sisson and Bronson⁶ have advocated gastric lavage with 20 per cent disodium phosphate dihydrate and intravenous administration of calcium disodium versenate. Blood volume and blood pressure are supported with solutions of glucose, saline or appropriate electrolytes.

Iron Deficiency Anemia in Patients With Cyanotic Congenital Heart Disease

In most infants and children with cyanotic congenital heart disease the bone marrow response to the persistent anoxic stimulus is polycythemia in which the hemoglobin increase approaches the rise in the erythrocyte count. Occasionally in patients the rise in hemoglobin does not keep pace with the erythrocytosis. Hemoglobin levels of 10 to 13 gm per 100 ml, erythrocyte counts of 6 to 8 million and hematocrit levels of 40 per cent accompanied by complaints of irritability, anorexia and poor weight gain have been observed in young patients.⁴⁴ Even hemoglobin levels of 16 to 18 gm per 100 ml may represent relative anemia in the presence of polycythemic blood levels. The administration of iron results in marked improvement in the clinical and hematologic status. Iron therapy is stopped or decreased when the hematocrit level reaches 75 per cent. Experience has shown that patients with appreciable arterial unsaturation function best with a hematocrit of 55 to 75 per cent. Patients with values lower than these require iron medication.⁴⁴

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Megaloblastic Anemia and Related Anemias

Megaloblastic anemias are characterized by a predominance of megaloblasts in the marrow as well as evidence of macrocytosis in the peripheral blood and are due to a deficiency of vitamin B₁₂ or folic acid. These two substances function as coenzymes in the synthesis of nucleoproteins and in their absence specific clinical and hematologic abnormalities develop.

Vitamin B₁₂ Vitamin B₁₂ is found principally in foods of animal origin such as liver, kidney, and muscle and to a lesser extent in eggs and milk. It is not usually present in higher plants but can be synthesized by certain bacteria among them *Streptomyces griseus*. It occurs also as a by-product of streptomycin. In its pure form vitamin B₁₂ is a crystalline substance containing cobalt whose chemical name is cyanocobalamin. It functions as "extrinsic factor" in regard to erythropoiesis and in the presence of the "intrinsic factor" of Castle secreted by gastric mucosa it is absorbed into the small intestine and passes into the blood stream to the liver where it is stored for use by the bone marrow and other tissues. The erythrocyte maturing factor (EMF) is probably identical with the extrinsic vitamin B₁₂ after its absorption. Vitamin B₁₂ exists in the serum mainly in combined form as a heat labile complex with serum protein specifically in the alpha globulin fractions.⁶ Normal serum has a limited capacity to bind crystalline vitamin B₁₂ in vitro.

Folic Acid Folic acid, also a member of the vitamin B complex, is distributed in plant and animal tissues chiefly in fresh leafy green vegetables such as spinach and cabbage and in liver, kidney, yeast, and mushrooms. Liver, beef, and fresh green vegetables are the common foods with a high folic acid content. Folic acid is readily absorbed from the gastrointestinal canal. In its synthesized form it is known as pteroylglutamic acid (PGA). For its metabolically active form it must be converted from its conjugated state to folinic acid (citrovorum factor) or to tetrahydrofolic acid.¹⁴

Formiminoglutamic acid (FIGLU), a normal intermediate in amino acid metabolism, requires the active tetrahydrofolic acid for its further degradation to glutamic acid. When tetrahydrofolic acid is insufficient, formiminoglutamic acid accumulates and its excretion in the urine thus serves as a useful indicator of clinical folic acid deficiency.^{18,22} Metabolic correction of the deficiency follows, a demonstration of folic acid can be shown by the elimination of the increased urinary formiminoglutamic acid.

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ogenesis of this disease. The occasional case in which a response is obtained with large doses of vitamin B₁₂ is explained by the sparing action on folic acid.¹⁸ Vitamin B₁₂ not only interacts with folic acid in the tissues as a coenzyme at different stages of nucleic acid synthesis but also influences in some way the storage and intake of folic acid.²⁰ A disorder identical with that in the human being has been produced in monkeys fed a diet largely of powdered milk notorious for its causative relationship to the disease in infants. Without additional ascorbic acid the animals develop scurvy and then megaloblastic anemia.¹⁹ On the other hand, the severe deficiency of folic acid as a complication of scurvy in monkeys fed milk diets is probably due to nonspecific factors operating in scurvy.¹

The participation of ascorbic acid in pathogenesis is emphasized by the fact that the incidence of megaloblastic anemia has dropped precipitously since infant foods have been fortified with large amounts of this vitamin. This dramatic change applied particularly to powdered milk preparations which had been low in ascorbic acid. There is reason to believe that many cases reported as goat's milk anemia are indeed megaloblastic anemia of infancy.

Clinical Features. The onset is insidious and usually makes its appearance between the ages of 2 and 17 months with a mean age of 7 to 8 months.¹⁹ Pallor, anorexia, irritability, failure to gain weight, persistent upper respiratory infections, intermittent fever and diarrhea are common. Petechiae, mucous membrane hemorrhages and ecchymoses in conjunction with thrombocytopenia are present in severely affected infants. The nutritional status is poor. The heart may be enlarged and some degree of hepatomegaly is a common feature. Splenomegaly is unusual. There is no neurologic component as in patients with pernicious anemia. There may be transitory gastric achlorhydria even after histamine administration and occasional hypoproteinemia.

Laboratory Findings. Anemia is mild or severe. Hemoglobin levels below 5 gm per 100 ml are common.⁴ The red blood cell count may be markedly reduced in occasional cases to less than 1 million per cubic millimeter. The blood smear shows anisocytosis, poikilocytosis and either a normochromic or hypochromic normocytic anemia. Occasional or moderate macrocytosis is noted. Except for leukocytosis with infections, leukopenia and neutropenia are common findings. The polymorphonuclear neutrophils are enlarged and hypersegmented. The platelets are reduced in number, often markedly so. In moderately or severely affected infants nucleated red cells appear in the peripheral blood. The bone marrow examination is diagnostic. The megaloblastic type of erythropoiesis corresponds to that of pernicious anemia. The open structure of the nucleus, its finely divided chromatin, easily visible parachromatin and basophilic cytoplasm characterize the megaloblast.³⁻⁵ Giant metamyelocytes and giant stab cells with distorted nuclei are pathognomonic of this disorder. Appearing at times in advance of megaloblasts, they are indications of the early stages of megaloblastic anemia. The variation in the number of megaloblasts, normoblasts and abnormal metamyelocytes in different cases indicate that the deficiency may be partial and not always complete. Formiminoglutamic acid urinary excretion has been found useful as an indicator of folic acid deficiency.¹⁴

Treatment. The anemia responds readily and completely to oral or parenteral

Folic acid deficiency results principally from inadequate dietary intake increased demand antagonists to its utilization (such as occur in antileukemia therapy) and interference with intestinal absorption. Occasionally, ascorbic acid deficiency contributes to the causation of megaloblastic anemia by limiting the conversion of folic acid to its active form.

Interrelationship of Folic Acid and Vitamin B₁₂ Folic acid is closely associated with vitamin B₁₂ in the synthesis of nucleic acids acting as catalysts at different stages of this process.¹ There is evidence also that a balance exists between folic acid and vitamin B₁₂ and that a deficiency of one increases the requirement for the other.^{2,40} Whereas a deficiency of either substance results in megaloblastic bone marrow and glossitis the nervous system disturbance of pernicious anemia for instance is caused solely by vitamin B₁₂ deficiency. The recovery from neurologic symptoms requires vitamin B₁₂ and not folic acid therapy. The deficiency of folic acid or vitamin B₁₂ not only results in the appearance of megaloblasts but also affects other cells in the bone marrow. The constituents of nucleic acids (uracil thymine and thymidine) possess therapeutic activity in patients with megaloblastic anemia but not to the extent that folic acid and vitamin B₁₂ do.³

The following megaloblastic anemias are due to the absence of gastric intrinsic factor or result from dietary deficiencies of either folic acid or vitamin B₁₂. Treatment consists of giving one or both of these factors depending upon the extent of the response.

- 1 Megaloblastic anemia of infancy
- 2 Juvenile pernicious anemia
- 3 Nutritional microcytic anemia
- 4 Impaired absorption from the gastrointestinal tract
 - A Sprue (tropical) idiopathic steatorrhea (nontropical sprue) celiac disease
 - B Congenital strictures gastrointestinal resections anastomoses
- 5 Miscellaneous
 - A Infestation with fish tapeworm
 - B Anticonvulsant drugs
 - (1) Phenytoin sodium (Dilantin)
 - (2) Primidone (Mysoline)
 - C Chronic liver disease
 - D Acute leukemia during treatment with antimetabolite drugs
 - E Sickle cell anemia and other hemolytic anemias
 - F Hemochromatosis

MEGALOBLASTIC ANEMIA OF INFANCY

Etiology Megaloblastic anemia of infancy is an acute anemia (rarely chronic) transitory in nature due to a deficiency of folic acid. It is brought on by states in which reserves of folic acid are greatly diminished such as during periods of rapid growth, high nutritional demands, infection, and prolonged diarrhea.^{9,40} Premature infants are especially susceptible. It is of interest that all types of milk used for infant feeding provide a low intake of folic acid and goats milk is especially poor in vitamin B₁₂, yet few infants develop the disease. Ascorbic acid and to some extent vitamin B₁₂ appear to play significant roles in the path

minished or absent gastric secretion of intrinsic factor. Histamine fast achlorhydria and a characteristic type of gastric atrophy^{4, 43} accompany the defective gastric secretion. Continuous treatment is required to maintain the patient in a state of remission. Without vitamin B₁₂ the erythroblast fails to mature and assumes the cytologic features of the megaloblast. The absorption of substances other than vitamin B₁₂ is usually normal. A familial tendency toward the disease has been noted.⁴⁴

In its complete form juvenile pernicious anemia is characterized as in the adult by a macrocytic anemia, megaloblastic bone marrow, atrophy of the papillae of the tongue and other features of recurrent glossitis, nervous system involvement, a specific response to parenteral injections of vitamin B₁₂ or liver extract converting the bone marrow to a normoblastic pattern and the need for continuous therapy to prevent relapses. Histamine refractory achlorhydria, one of the cardinal features of adult pernicious anemia is an inconstant finding, however, in the juvenile form of the disease.

Clinical Manifestations. Many of the cases are detected during the first two years of life, whereas others become manifest during childhood until puberty. The onset is insidious and pallor, apathy, fatigability, anorexia and diarrhea are early symptoms. A beefy red sore tongue, papillary atrophy and other features of recurrent glossitis are common. Signs of subacute dorsolateral degeneration of the spinal cord occur infrequently.⁴⁵ Most common are neurologic signs including ataxia, less often paresthesia of the hands and feet, impaired vibratory perception, Babinski signs and absence of tendon reflexes. Jaundice is absent and there is no enlargement of the liver and spleen.

Laboratory Findings. Microcytes, many of them oval, anisocytosis, poikilocytosis, polychromasia and occasionally nucleated red cells characterize the blood smear and parallel the severity of the anemia. The hemoglobin ranges from 4 to 9 gm. per 100 ml., usually below 6 gm. The red blood cell count is comparatively lower than the hemoglobin level, varying from 1 to 2.5 million per cubic millimeter.

The anemia, being macrocytic, shows a high color index and an increase in the mean corpuscular volume (MCV) ranging from 110 to 140 cubic microns, which is well above the upper limit of normal of 92 cubic microns. The life span of the red cells is moderately shortened.⁴⁶ The macrocyte, normally saturated with hemoglobin, takes a dark stain and conveys the impression of hyperchromia. Supersaturation is nonexistent. Leukopenia is common and may be marked with the number of white cells ranging from 3,000 to 4,000 per cubic millimeter and is caused by a depression of myeloid tissue in the bone marrow. Hypersegmentation of oversized polymorphonuclear leukocytes (macropolycytes) with bizarre shaped lobes is common. The platelets are reduced in number. The leukopenia and thrombocytopenia are not so marked as to lead to susceptibility to infection or purpura.

The bone marrow is hyperplastic with the nucleated red cells ranging up to 50 per cent of the total cells. Of these the megaloblasts in different stages of maturation and hemoglobination predominate.⁴⁷ Mitotic figures are common. The abnormally large size of the polymorphonuclear cells and the metamyelocytes

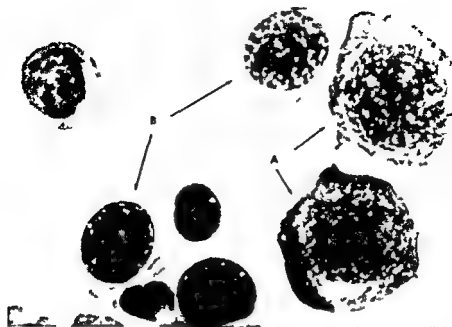


Fig 8 Bone marrow smears from a patient with megaloblastic anemia of infancy showing megaloblastic erythroid precursors ($\times 900$). Note the more typical megaloblastic chromatin structure in the early myeloblasts A and the less well defined changes in the normoblasts B. Cell at upper left is a small lymphocyte. (From Lubin L. Megaloblastic Anemia in Infancy. *J Pediatr* 54:617, 1959.)

administration of folic acid given daily in a dosage of 10 to 30 mg over a period of two to three weeks or until the blood and bone marrow picture returns to normal. A reticulocyte rise follows the administration of folic acid. Complete disappearance of the megaloblastic pattern is noted two days after the injection of folic acid.⁴ Liver extract is of value but it is not so direct or reliable as folic acid. Ascorbic acid should be given simultaneously in a dose of approximately 200 mg if there is any suspicion of its deficiency. Some workers advocate a combination of folic acid and ascorbic acid from the outset as a routine procedure. In countries where malnutrition and poverty exist and megaloblastic anemia is prevalent, vitamin B₁₂ is also added.¹ An enriched diet containing folic acid and vitamin B₁₂ is essential. Antibiotic therapy is important especially since infection plays such an important role in etiology. Transfusions are indicated when anemia is severe but folic acid must be given at the same time.

Prognosis. Complete recovery within a few weeks is expected following folic acid therapy. Although the administration of folic acid produces a permanent cure of megaloblastic anemia of infancy, juvenile pernicious anemia with a similar megaloblastic bone marrow is a lifelong disease requiring continuous therapy.

JUVENILE PERNICIOUS ANEMIA

Etiology. Pernicious anemia in childhood is exceedingly rare. Addisonian pernicious anemia is a megaloblastic anemia caused by a deficiency of vitamin B₁₂ which is based on a defective absorption of the vitamin resulting from di-

diminished or absent gastric secretion of intrinsic factor. Histamine fast achlorhydria and a characteristic type of gastric atrophy^{4, 5} accompany the defective gastric secretion. Continuous treatment is required to maintain the patient in a state of remission. Without vitamin B₁₂ the erythroblast fails to mature and assumes the cytologic features of the megaloblast. The absorption of substances other than vitamin B₁₂ is usually normal.⁶ A familial tendency toward the disease has been noted.^{7, 8}

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Laboratory Findings Macrocytes many of them oval anisocytosis poikilocytosis polychromasia, and occasionally nucleated red cells characterize the blood smear and parallel the severity of the anemia. The hemoglobin ranges from 4 to 9 gm per 100 ml usually below 6 gm. The red blood cell count is comparatively lower than the hemoglobin level varying from 1 to 25 million per cubic millimeter.

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are characteristic features. The changes are particularly pronounced among the metamyelocytes which are giant in size, possess a U shaped nucleus and have a bulky, poorly granulated cytoplasm.

Diagnosis Because of the rarity of pernicious anemia in children it is important to eliminate other causes of megaloblastic anemia. The difficulty in diagnosis has been the presence of free hydrochloric acid in the gastric contents and the inconsistency of histamine refractory achlorhydria in many of the reported cases.¹¹ Long term follow up is necessary to determine whether achlorhydria essential for the diagnosis of the adult disease will eventually develop. The presence of a megaloblastic bone marrow, the absence of intrinsic factor, a good reticulocyte response following the administration of vitamin B₁₂, and the continuous requirement of vitamin B₁₂ to maintain a remission of the disease constitute the basic diagnostic features.

A young man who was reported to have had pernicious anemia at the age of 13 months was found to be still suffering from vitamin B₁₂ deficiency at 18 years of age.¹² He secreted normal amounts of acid and pepsin in the gastric juice but was unable to absorb a test dose of radioactive vitamin B₁₂ unless this was given with a source of intrinsic factor. The gastric juice was deficient in intrinsic factor but the gastric mucosal membrane was histologically normal. The patient's father suffered from typical Addisonian pernicious anemia. It was suggested that vitamin B₁₂ deficiency itself could cause temporary gastric atrophy which becomes permanent and complete if treatment is withheld too long. In another study¹³ two children with pernicious anemia showed normal gastric function and structure. An inability to absorb vitamin B₁₂ from the gastrointestinal tract was demonstrated. The author postulated that the gastric manifestations of pernicious anemia are not an intrinsic part of the disease but are secondary to chronic deficiency of vitamin B₁₂.

A number of tests have been devised for the confirmation of the diagnosis of pernicious anemia.¹⁴ The newer methods include biopsy of the gastric mucosa¹⁵ to determine the presence of atrophic changes, estimation of serum vitamin B₁₂ level and methods for determining the amount of radioactive vitamin B₁₂ absorbed from the intestine. The urinary excretion method of Schilling¹⁶ one of the simplest consists of the oral administration of a small dose of radioactive vitamin B₁₂ followed by a flushing dose of 1 mg. of nonradioactive vitamin B₁₂ injected parenterally immediately or within two hours. Following the oral dose of radioactive vitamin B₁₂ substantial amounts of the vitamin are excreted in the urine of normal persons within twenty four hours as compared with minute amounts in patients with pernicious anemia. In patients with pernicious anemia serum vitamin B₁₂ levels are low.

Course and Prognosis With modern treatment utilizing vitamin B₁₂ in adequate dosage the outlook for reversal of the disorder is excellent. Infections and complications arising from nervous system involvement interfere with treatment.

Treatment The response to liver extract and vitamin B₁₂ is rapid and specific. The standard form of treatment consists of the parenteral injection of vitamin B₁₂. The dosage is to some degree an individual matter. In children a remission of the disease should be obtained with doses of 15 to 30 micrograms given every day or two for two to four weeks followed by a maintenance dose of the same amount given every two to four weeks. Large doses may be required. An alternate schedule is to give a large initial dose daily for 5 injections or every second

on the third day for ten to fifteen days followed by 1 injection of 100 micrograms each month.⁹ Liver extract may be given on the basis of its vitamin B₁₂ content.

The reticulocytes begin to increase on the third to fourth day, rise to a maximum on the sixth to eighth day, and fall gradually to normal about the twentieth day.⁹ The height of the reticulocyte count is inversely proportional to the degree of anemia. Beginning bone marrow reversal from megaloblastic to normoblastic cells is obvious within six hours and is completely normoblastic in seventy-two hours.

Prompt hematologic responses are also obtained with the use of oral folic acid. Folic acid has the disadvantage of having no effect upon neurologic manifestations and has been known to precipitate or accelerate their development. Iron is occasionally required when a generally inadequate diet has been given which is deficient in this mineral.

NUTRITIONAL MEGALOBLASTIC ANEMIA

Nutritional megaloblastic anemia is a macrocytic anemia occurring among underprivileged peoples in tropical and subtropical countries where malnutrition is prevalent due to poverty and dietary habits.⁴¹ It results from an inadequate dietary intake of folic acid and vitamin B₁₂. The megaloblastic bone marrow macrocytic anemia and leukocytic changes respond to folic acid with or without vitamin B₁₂.¹ Correction of dietary defects is essential. Iron and transfusions are given if needed.

This type of anemia is to be differentiated from that occurring in kwashiorkor. The latter, originally described as a nutritional syndrome of African infants and children, is also prevalent in Central and South America,³ India, China, and other countries and results from severe protein deficiency. The intake of calories derived largely from starchy food is adequate. The anemia is usually normochromic and normocytic and less commonly macrocytic and microcytic.¹ Occasionally it is aggravated by blood loss due to hookworm and malaria. The bone marrow is normoblastic or megaloblastic.^{1,2} The anemia of kwashiorkor has also been found in association with aplastic crises (erythroblastopenia) in which giant proerythroblasts appeared in the marrow.¹⁶ Patients with the normochromic normocytic type of anemia respond to a well balanced diet rich in protein but not in liver extract, folic acid, or vitamin B₁₂. Iron is occasionally effective. In those cases of kwashiorkor in which megaloblastic anemia of infancy is diagnosed, the bone marrow reverts to normal following the administration of folic acid.³⁹

Nutritional megaloblastic anemia of pregnancy is also more common in underprivileged areas and is due to a chronic dietary deficiency. The prognosis for the mother and fetus has improved with the use of folic acid and vitamin B₁₂.⁴¹

MISCELLANEOUS MEGALOBLASTIC ANEMIAS

Megaloblastic Anemia With Hemolytic Anemias

Megaloblastic anemia is a rare complication of hemolytic anemia and has been reported in association with acquired hemolytic anemia, hereditary spherocytosis,

thalassemia, sickle cell anemia and paroxysmal nocturnal hemoglobinuria. The megaloblastic bone marrow is attributed to folic acid deficiency caused by the demands for this substance imposed by rapidly developing erythroid cells. The need for folic acid exceeds the dietary intake and results in tissue depletion.

Megaloblastic Anemia With Hemochromatosis

The occurrence of a microcytic anemia with a megaloblastic type of erythropoiesis has been observed in hemochromatosis. Its causation as well as its formation and absorption is related to the associated pigmentary cirrhosis hindering the storage of anti-anemia factors. In some patients liver therapy has been effective¹ in one patient there was a striking response to folic acid but not to vitamin B₁₂.²²

Megaloblastic Anemia With Fish Tapeworm

The fish tapeworm (*Diphyllobothrium latum*) leads to megaloblastic anemia indistinguishable from pernicious anemia. This type of anemia is caused by competition of the tapeworm with the host for the vitamin B₁₂ in the food in the intestinal tract necessary for the nutrition of the parasite.²³ Treatment depends upon expulsion of the parasite or the administration of liver or vitamin B₁₂.

Megaloblastic Anemia Caused by Anticonvulsant Therapy

Extremely rare causes of megaloblastic anemia are the anticonvulsant agents phenytoin sodium (Dilantin)²⁴ and primidone (Mysoline).²⁵ The corrective effect of folic acid suggests that phenytoin and primidone may act as competitive inhibitors of some enzyme system normally involving folic acid.²⁶ A conditioning factor for the development of anticonvulsant megaloblastic anemia may be the presence in the patient of occult nutritional deficiency secondary to an inadequate diet particularly of folic acid and to some extent of vitamin C.

MALABSORPTION SYNDROMES—SPRUE (TROPICAL) IDIOPATHIC STEATORRHEA (NONTROPICAL SPRUE) AND CELIAC DISEASE

Except for the common factor of impaired motility and absorption of the small intestine there are no specific anatomic or pathologic changes in patients with sprue, idiopathic steatorrhea or celiac disease. These diseases show a marked variation with regard to clinical features and causation.²⁷ Although the passage of stools containing an excess of fats (steatorrhea) is a characteristic feature of all of these diseases the fecal content of other nutrients is also increased. Thus the body loses vitamins, minerals, proteins, carbohydrate and such hemitopoietic substances as folic acid, vitamin B₁₂ and iron. Diarrhea, weakness and wasting are prominent symptoms.

Tropical sprue has a seasonal incidence and is limited largely to certain overcrowded and underdeveloped geographic areas in tropical and subtropical climates. It develops abruptly and runs a rapid course. Whether it is primarily or only secondarily a deficiency disease is not known.²⁸

Idiopathic steatorrhea (nontropical sprue) is less common, occurs sporadically, has no seasonal or geographic incidence, and is usually a chronic and insidious disease.⁶

Celiac disease is included by some authors with nontropical sprue since it may persist into adult life. Celiac disease (Gee Herter disease) is a chronic disorder of infants and children, is characterized by diarrhea, wasting (especially noticeable in the flattened buttocks and groins), retardation of growth, and abdominal distention. The proteins (glutens) of wheat and rye are precipitating factors of the disorder; the gliadin fractions acting as toxic rather than allergic agents.³⁷

In most cases of tropical sprue and macrocytic anemia indistinguishable from Addisonian pernicious anemia is present and is associated with a megaloblastic bone marrow. Although a macrocytic anemia is commonly found in patients with nontropical sprue, it only occasionally presents the characteristics of pernicious anemia and may not be associated with megaloblastic bone marrow.⁶ Glossitis is present in patients with tropical and nontropical sprue. Folic acid, vitamin B₁₂, and liver extract are specific therapeutic agents in the treatment of patients with tropical sprue with megaloblastic anemia. The deficiency pattern including diarrhea takes longer to reverse. Folic acid, vitamin B₁₂, and liver extract are also helpful in patients with nontropical sprue with megaloblastic anemia, although hematologic remission is not always complete and there is no demonstrable effect on the steatorrhea. Hypochromic microcytic anemia is by far more common than the macrocytic type in celiac disease. Iron administered orally or parenterally is given for the iron deficiency, and folic acid, liver extract, and vitamin B₁₂ are given for the macrocytic anemia.

The megaloblastic anemia associated with intestinal strictures, fistulas, and anastomoses is in some way dependent upon the invasion of the small intestine by organisms normally inhabiting the colon. In fistulas such as the ileocolic variety, there is a direct contamination by organisms from the colon. In strictures and blind loops, stagnation predisposes to abnormal bacterial growth and absorption of toxic substances.^{4,38} Most of the clinical and hematologic features are those of pernicious anemia or tropical sprue. Vitamin B₁₂ and especially folic acid have a beneficial effect on the anemia. Surgical correction of the intestinal disorder is often followed by relief of the anemia, but long-term parenteral vitamin B₁₂ administration may still be necessary. When intestinal stasis is a factor in the course of intestinal stricture and anastomosis, the impaired absorption of vitamin B₁₂ may be significantly improved by administration of the tetracycline group of antibiotics.¹⁴

NORMOBLASTIC MACROCYTIC ANEMIAS

The normoblastic macrocytic anemias constitute a heterogeneous group in which the bone marrow is normoblastic but macrocytes appear in variable numbers in the peripheral blood interspersed among the red cells of a normocytic normochromic anemia. These macrocytic anemias are secondary to aplastic anemia, lymphomas, and primary nonhematopoietic diseases such as hypothyroidism, scurvy, and liver disease.

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Hypoplastic and Aplastic Anemias

General Considerations The hypoplastic and aplastic anemias constitute a heterogeneous group of anemias which include several well defined entities and a larger number of still obscure hypoplastic states reflecting degrees of erythropoietic suppression. These anemias are not caused by excessive hemolysis, blood loss, nor in the majority of instances by space occupying infiltrations of the bone marrow. The cardinal feature of this group of anemias is bone marrow hypofunction resulting from a failure of red cell production and hemoglobin synthesis to compensate for the normal rate of blood destruction. Erythropoiesis alone may be significantly decreased or the defect may also extend to the granulocytes and platelets resulting in a peripheral blood picture of pancytopenia. Bone marrow aspiration or biopsy reveals either the anticipated aplasia or a specimen varying from normal cellularity to hypercellularity in which case depressed functional activity is postulated.¹⁰ The difficulties of reconciling the state of the bone marrow with the peripheral blood picture and the resistance of the hypoplastic/aplastic group to all forms of intravenous therapy except transfusion have prompted their designation as refractory anemias.³ Abnormalities in the metabolism of nucleic acids particularly DNA have been postulated as a cause of many refractory anemias in which peripheral pancytopenia is accompanied by a cellular hyperplastic bone marrow.^{10a}

The anemias are usually normochromic and normocytic; occasionally macrocytic with moderate anisocytosis and are distinguished from other blood dyscrasias by a diminished number of reticulocytes and other evidences of ineffective blood formation. The absence of enlargement of lymph nodes, liver and spleen in patients with the major anemias of this group contributes a feature of diagnostic importance.

Classification For purposes of simplification these anemias have been consolidated into two general categories—the hypoplastic and aplastic anemias. Although overlapping obviously occurs there are members of each group nevertheless which possess such well defined characteristics as to constitute specific entities.

Aplastic anemia is characterized by a simultaneous depression of the three principal cellular elements in the bone marrow and a peripheral picture of profound anemia, leukopenia, neutropenia and thrombocytopenia. It can be noted from the accompanying classification that aplastic anemia includes both primary

and secondary forms although the former are more prominent in infancy and childhood

Hypoplastic anemia differs from aplastic anemia in that the formation of red cells is impaired, with lesser or no involvement of granulocytes and platelets. In recent years the term hypoplastic anemia has been applied rather loosely to intermediate or miscellaneous conditions. Reports of cases of hypoplastic anemia now range from conditions in which there is a failure of red cell production to those in which leukocytes and platelets are simultaneously depressed but to a lesser degree than red cells. This tendency has been exaggerated by the knowledge that a transient anemia accompanied by a diminished reticulocyte count need not necessarily be correlated with a bone marrow deficient in erythroid tissue. To one of the members of this group pure red cell anemia, a restrictive connotation should be applied. This is a chronic congenital aregenerative anemia which had been previously placed into a separate category⁴ but which is now classified with the hypoplastic anemias in contrast to the acquired forms of erythroblastopenia.

- I Hypoplastic anemia (major effect on erythropoiesis)
 - A Idiopathic—hypoplasia confined to erythropoiesis
 - (1) Congenital—pure red cell anemia (chronic congenital aregenerative anemia)
 - B Acquired—with varying degrees of erythrocytic hypoplasia
 - (1) Anemias due to infections drugs chemicals toxins autoimmune and allergic states
 - (2) Aplastic crisis in course of hemolytic syndromes such as hereditary spherocytosis and sickle cell anemia
 - (3) Anemia resulting from suppressive effect of multiple transfusions on erythropoiesis
 - (4) Miscellaneous—moderate anemia with or without lesser involvement of granulocytes and platelets
- II Aplastic anemia (severe pancytopenia)
 - A Idiopathic or primary
 - (1) Congenital aplastic anemia
 - (a) With multiple congenital anomalies (Fanconi type)
 - (b) Without associated anomalies
 - (2) Nonfamilial and without associated anomalies
 - B Acquired—secondary
 - (1) Anemia due to antimicrobial and other chemotherapeutic agents
 - (2) Anemia due to industrial and household chemicals
 - (3) Anemia due to irradiation
 - (4) Miscellaneous causes—infections endocrine factors bone marrow invasion or replacement (myelophthisic anemias) hypersplenism

HYPOPLASTIC ANEMIAS

Idiopathic Hypoplastic Anemia—Pure Red Cell Anemia

Definition Pure red cell anemia is also called chronic congenital aregenerative anemia, congenital hypoplastic anemia, pure red-cell aplasia, primary erythroid hypoplasia, erythrocytogenesis imperfecta, etc. The term pure red cell anemia⁵ refers to a chronic progressive anemia confined to a failure of erythropoiesis without an equivalent depression of the white blood cells or platelets.^{6,7} The

bone marrow is characterized by a complete or almost complete absence of cells of the erythroid series with normally proliferating cells of the granulocytic series and megakaryocytes. The failure of the bone marrow to provide adequate numbers of erythrocytes results in a persistent and progressive anemia with the need for long term supportive transfusions. Pure red cell anemia has been observed at all ages but it is particularly conspicuous as a congenital disease in early infancy and childhood.

Pathogenesis The etiology is unknown but several theories have been advanced to explain a condition in which only a single element of the bone marrow is affected. It is conceivable that the hematopoietic defect results from a noxious factor operating at critical periods of fetal development. Pure red cell anemia has been described in association with other development defects such as a hypoplastic and cystic kidney⁴³ and complete¹⁰ and temporary¹⁸ pure red cell anemia has been reported in patients with autoimmune hemolytic disease. Pure red cell anemia may conceivably result from a similar mechanism—namely, the action of such antibodies against the red cells in fetal life producing prolonged and permanent depression of erythropoiesis.⁴⁴ Pure red cell anemia may be compared with other congenital anomalies caused by developmental defects.

Anthranilic acid, a metabolite formed in the breakdown of tryptophan, is excreted in relatively large quantities when a riboflavin deficient animal is fed tryptophan. A similar defect in tryptophan metabolism has been reported in a number of patients with congenital pure red cell anemia.¹ The administration of riboflavin to these patients resulted in a decrease in the excretion of anthranilic acid but did not alter the hematologic status.

Clinical Features Pure red cell anemia has an insidious onset with progressive pallor, anemia, irritability, listlessness, and anorexia which are usually apparent at the age of 2 to 3 months or later in the first year. The infant appears healthy at birth and general development proceeds normally. Exceptionally the onset of the disease may be delayed; such a delay has been reported in a child 11 years old⁹ and two brothers 17 years of age.⁸ This occurrence in siblings has also been noted in other cases.³

Except for pallor, there is little to be observed at physical examination. The liver, spleen, and lymph nodes are not enlarged; jaundice is absent and there are no manifestations of bleeding. In some patients a particular type of facies has been described,⁴ consisting of tow-colored hair, snub noses, thick upper lips, rather wide-set eyes, and an intelligent expression.

The liver and spleen enlarge as transfusion hemosiderosis develops. Normal red cell survival which is present when the spleen is not enlarged is shortened as this organ becomes the site of increased red cell destruction. Long-standing administration of blood results in increased pigmentation of the skin. This is due to both iron deposition and excessive melanin formation.⁶ Cardiac enlargement, multiple arrhythmias, and refractory heart failure are frequently inevitable consequences.

Pure red cell anemia also occurs as an acquired erythrocytic hypoplasia in adults without a proved common etiology.^{41, 45} Infection, allergy, red cell auto-antibodies in hemolytic anemia, and chemical exposure have been incriminated.

A more constant finding is the coexistence of this disease with benign thymoma⁸ ■ 339

Laboratory Findings The outstanding feature is a chronic aregenerative and refractory anemia in which there is a striking absence from the marrow of the nucleated precursors of the red cells without a simultaneous depression of granulocytes platelets and their precursors. The anemia is normocytic and normochromic with reticulocytes low in number or absent. Without supportive transfusions the hemoglobin falls to extremely low values.

The number of platelets white blood cells and differential counts are normal. Marked leukopenia and moderate depression of platelets occur in rare instances in which the spleen enlarges and manifests a hypersplenic function. The bone marrow in such a patient shows normal myeloid activity and a normal number of megakaryocytes. In a boy 7½ years old under observation splenectomy resulted in a return of leukocytes and platelets to normal but erythropoiesis remained hypoplastic. The serum iron level is elevated with complete saturation of the latent iron binding capacity as a result of prolonged transfusion therapy.

In this condition also the bone marrow frequently contains varying numbers of primitive cells termed hematogones.⁴³ Although such cells usually are smaller than the ordinary lymphocytes they resemble them closely in size and morphology. They possess a dense homogenous matlike nucleus and a narrow rim of nongranular basophilic cytoplasm in which lymphocytes are usually entirely absent. These primordial cells possibly represent progenitors of the erythrocytic series. In one patient they were replaced by small numbers of normoblasts following the administration of cortisone.

Pathologic Findings The essential feature is widespread hemosiderosis at times associated with evidence of hemochromatosis. In a 10 year old patient who died of heart failure postmortem examination revealed the typical syndrome of hemochromatosis including diabetes mellitus and skin pigmentation. The heart was greatly enlarged and many of the myocardial fibers contained iron pigment scanty interstitial fibrosis and occasionally necrosis of isolated fibers. In a 9 year old child whose terminal illness was severe hepatitis postmortem examination revealed massive liver necrosis and generalized hemosiderosis with fibrosis of the liver pancreas and thyroid. The myocardial fibers were hypertrophied vacuolated and contained moderate amounts of hemosiderin.

Diagnosis In a typical congenital case the disease is fully established in early infancy. The need for frequent transfusions is soon found to be based on erythropoietic hypoplasia in the bone marrow. Occasionally pure red cell anemia may follow erythroblastosis and be mistaken for the protracted depression of erythropoiesis which sometimes accompanies the latter disease. Pure red cell anemia can be differentiated from the hemolytic anemias such as sickle cell disease thalassemia major and hereditary spherocytosis by the lack of splenomegaly and the absence of icterus reticulocytes and morphologic abnormalities in the red cell.

There is as yet no unanimity as to the degree of erythroid hypoplasia which the name of this anemia implies. Although the designation of pure red cell anemia relates to conditions in which the bone marrow is practically devoid of normoblasts (less than 2 per cent) in some reports these elements though

reduced are nevertheless present in moderate numbers. The myeloid erythroid ratio which should be necessarily high in a typical case may be only moderately reduced in others. In one report¹ the disorder is referred to as "erythrogenesis imperfecta" in which several bone marrow examinations showed a variation between erythroid hypoplasia and hyperplasia. These cases however share the common feature of varying aplasia of erythropoiesis without involvement of leukocytes or platelets. The differentiation from aplastic anemia and the acquired erythroblastopenia will be discussed subsequently.

Treatment Major therapy consists of transfusions, ACTH and steroids and splenectomy.

Transfusions Supportive transfusions to maintain the hemoglobin at levels compatible with freedom from symptoms constitute a basic need. The use of sedimented or packed cells is preferable to whole blood. As a guide to management we found that with few exceptions patients in this category of chronic anemia do not require transfusions until hemoglobin levels drop to 7 to 7.5 gm per 100 ml at which point clinical symptoms usually appear. These include anorexia, listlessness, apathy and incipient signs of heart failure. It is apparent that hemosiderosis is to be expected following repeated transfusions. Another limitation of frequent transfusions is their potential depressant effect upon endogenous erythropoiesis and hemoglobin synthesis.¹⁸

ACTH and Steroids In association with transfusions adrenocortical hormones and ACTH are given primary consideration since they represent a potent form of therapy.^{1, 11, 13} Depending upon the age of the patient the steroid hormones (prednisone, prednisolone and like synthetic substances) are prescribed in a daily dosage of 15 to 60 mg given in divided doses every six hours (a total of 15 to 20 mg in children under 2 years of age and up to 60 mg in older children). With optimal dosages evidences of the ability of the bone marrow to produce red cells should be manifested within three to four weeks. If and when the patient responds the dosage should be reduced to the minimum at which a remission is maintained (approximately 15 mg daily in divided doses).

A favorable response is reflected in a bone marrow and blood remission—with erythroid hyperplasia, reticulocytosis and increased hemoglobin and red cell levels. Any evidences of infection should prompt the administration of a suitable antibiotic. Despite the persistently low hemoglobin levels these patients are not unduly susceptible to infection. If no response is obtained with the steroids in the prescribed period ACTH by intramuscular or subcutaneous injection should be substituted preferably as the gel in a daily dosage of 40 mg in the older child. Here too the response varies with each patient so that no fixed amount can always be prescribed. With a favorable response reducing and stopping the drug entirely should be attempted periodically to determine the capacity of the patient to establish spontaneous erythropoietic function.

It has been recommended that steroids be given early in the course of treatment since no response is to be expected when hemosiderosis has resulted after prolonged transfusion therapy.¹³ In our experience only one of six patients with congenital pure red cell anemia responded favorably to steroid therapy—in a 17 month old patient. In another patient of equal age in this group steroid treatment

was ineffective. In the remaining four patients steroids were given at various intervals during the course without effect. In another series¹ on the other hand ten patients responded favorably. Intermittent corticosteroid therapy was recommended to obviate growth and bone age retardation and osteoporosis. It was postulated that the mechanism of action was stimulation of an enzyme system possibly tryptophan oxidase. Reticulocytosis occurred at about nine days following treatment with a peak response of 12 to 18 per cent between fourteen and twenty two days. Most patients had a normal hemoglobin level within six weeks and two patients maintained this improvement without further treatment. Allen and Drimond¹ caution the need for early treatment since none of the patients who had the disease for more than three years responded whereas all children who had disease for three months or less did respond.

Splenectomy. Splenectomy which is frequently recommended only as a last resort in the treatment of pure red cell anemia has nevertheless proved effective on occasion both in reducing the number of transfusions and in restoring erythropoiesis.^{10, 9} When transfusions are required at increasingly frequent intervals to maintain a physiologically sufficient although reduced hemoglobin level it is postulated that an extracorporeal hemolytic component has developed which is presumably located in the spleen. Accelerated destruction can be confirmed by tagging normal donor red cells with radioactive chromium and noting their shortened survival.

In one of our patients with pure red cell anemia marked enlargement of the spleen was present. This finding is contrary to an accepted criterion of the aplastic hypoplastic group of anemias—namely the failure to palpate the spleen. In this patient however removal of the spleen resulted in extending the interval between transfusions although depression of erythropoiesis persisted. Undoubtedly in patients without enlargement splenectomy is justified when a hemolytic factor for donor blood is demonstrated by techniques revealing a shortened survival of transfused normal erythrocytes. The presence of such a hemolytic factor would be clinically suspected when regularly spaced intervals for transfusions are suddenly shortened.

In patients in whom no hemolytic component is apparent splenectomy is considered on the basis that an abnormally functioning spleen may depress the erythropoietic function of bone marrow. This procedure is recommended when ACTH, the steroids and other therapeutic agents alone or in combination have been given extensive trial without success. There is less reluctance to remove the spleen when the bone marrow is not entirely devoid of erythroid elements in any stage of development. In any event the procedure may be justified on the basis of a splenic humoral factor which inhibits maturation of precursors either visualized or potentially present in unidentified primordial cells such as hematogones. The fear that removal of the spleen deprives the patient of needed resources of erythropoietic tissue is not justified by results in patients with this disease. Although the majority of patients are not improved by splenectomy the condition is not aggravated. Occasionally splenectomy alone has a beneficial effect in restoring erythropoiesis. In other patients cortisone and other therapeutic agents have proved more effective after splenectomy. Increased

experience may show that splenectomy will be efficacious in patients who respond initially to steroid therapy.⁹

Prognosis In most patients the course of congenital pure red cell anemia is one of chronic and progressive anemia with a fatal outcome. In selected patients supportive transfusion therapy, ACTH and steroids and splenectomy can maintain a concentration of hemoglobin compatible with health. Long term transfusions however carry the hazards of hemosiderosis and hemochromatosis. Reported cases suggest that a more favorable response to steroid therapy could be expected in patients with moderate numbers of normoblasts in the bone marrow than in those in whom the normoblasts were practically depleted. In one of our patients however no response with corticosteroids was obtained despite an initial finding of 5 per cent normoblasts.

An important factor in evaluating therapy and in a consideration of prognosis is the possibility of spontaneous recovery. Such remissions occurred in three of twelve patients cited by Diamond⁷ and in others such as those reported by Palmen and Vahlquist.³¹ Remission can occur at any period from early childhood to adolescence with stabilization at a lower hemoglobin level.

Death results from cardiac failure, hepatitis during the course of transfusions and overwhelming infection and sepsis. In these terminal events the factor of hemosiderosis from excessive iron deposition resulting from transfusions and hemochromatosis may conceivably play an important complicating role.

Acquired Hypoplastic Anemias

Anemia Due to Infections, Drugs, Chemicals, Toxins, and Autoimmune and Allergic States Under the heading of acute erythroblastopenia, Casser¹⁸ described temporary regenerative crises of one week's duration resulting in severe anemia in children as a result of toxic, infectious or allergic causes. This acquired type of pure red cell anemia was noted in patients who had been given drugs such as amidopyrin, salicylates and barbiturates in those with infections including mumps, atypical pneumonia, bacterial sepsis due to meningococcus and staphylococcus and in those with autoimmune hemolytic disease.¹⁹

Aplastic Crisis The aplastic crisis occurring in patients with a number of hemolytic conditions, notably hereditary spherocytosis has been discussed elsewhere. Crises in these conditions are not hyperhemolytic as originally supposed but are aplastic. The bone marrow shows a complete absence of erythropoiesis, the occasional presence of proerythroblasts, a shift to the left of the granulocytic series and a decrease of megakaryocytes. The severity of the anemia is aggravated by the fact that the aplastic state occurs in patients with pre-existing hemolytic disease in which the life span of the red cell is already reduced.

Anemia Resulting From Suppressive Effect of Multiple Transfusions on Erythropoiesis The suppressive effect of multiple transfusions on erythropoiesis which has been demonstrated in connection with chronic hemolytic anemias¹⁶ probably extends also to the refractory anemias in which transfusions are the mainstay of treatment.

Miscellaneous The miscellaneous group of hypoplastic anemias is by far

the most common. Persistent ill defined anemias in children are often undiagnosed or are suspected of being in intermediate stage of idiopathic aplastic anemia. The normochromic and normocytic anemia which is found in accordance with the bone marrow which is normal both with respect to total cellularity and in the content of erythroid elements. The anemia is moderately severe, resists treatment with iron and other hematinics and may be corrected periodically by the administration of blood. Not until the basic difficulty, such as a specific hypersensitivity or the source of chronic upper respiratory infections for instance, is eliminated or treated adequately will the hemoglobin level return to normal.

APLASTIC ANEMIA (BONE MARROW FAILURE REFRACTORY ANEMIA)

Definition Aplastic anemia refers to a refractory anemia characterized by severe anemia, leukopenia and thrombocytopenia and is usually associated with aplasia or hypoplasia of the bone marrow. Ehrlich is credited with the first description of the disease in a young adult in 1885.⁹ The term aplastic anemia gained popularity as increasing numbers of cases with pancytopenia, often in the absence of a hypoplastic marrow, were reported.

Etiology The search for a history of exposure to agents injurious to the hematopoietic system is often difficult to obtain in children so that the majority of cases of aplastic anemia in younger age groups are eventually classified as idiopathic.

In a series of 334 patients with aplastic anemia Wolff¹⁰ found 24.3 per cent under the age of 15 years. Of the total number of patients 57.2 per cent gave no history of possible exposure to a toxic compound and were classified as having idiopathic anemia, 23.6 per cent had been exposed to one or more antineoplastic agents shortly before the onset of the disease, 9 per cent had had an exposure to other types of possible toxic materials and 10.2 per cent gave a combined exposure history. In another series Scott and co-workers¹⁰ found that seventeen of thirty nine patients (thirteen below 20 years of age) could give no history of exposure to known or suspected marrow depressants and were therefore considered to have the idiopathic disease.

Aplastic anemia is classified as either idiopathic or secondary, caused by a drug, chemical or ionizing radiation. It is possible that patients who give no history of toxic exposure and are classified as having idiopathic anemia might well have been exposed to an unrecognized agent. In some patients an interval of several weeks may elapse following exposure to a toxic agent before clinical signs and symptoms appear. Even with a reliable history of exposure the relationship between the toxic agent and bone marrow depressant may have been fortuitous. Idiopathic and secondary cases of aplastic anemia have similar clinical pictures except that patients with the secondary type have a somewhat better prognosis.

Idiopathic Aplastic Anemia

Included among the cases of bone marrow failure of unknown etiology are two distinctive syndromes—one associated with congenital anomalies and the other without anomalies.

Congenital Aplastic Anemia With Multiple Congenital Anomalies (Fanconi Type) The best known group of congenital malformations in which anemia constitutes an outstanding symptom is represented by the Fanconi syndrome. This condition includes the rare association of pancytopenia, bone marrow hypoplasia and a number of congenital anomalies—namely a patchy brown pigmentation of the skin due to a deposition of melanin, hypogonadism, microcephaly, dwarfism, strabismus, ptosis of the eyelids, nystagmus, exaggerated deep tendon reflexes, mental retardation, anomalies of ears, deafness, and skeletal abnormalities. Anomalies of the thumb, radii, long bones, congenital dislocation of the hips, syndactyly, congenital heart disease, and kidney anomalies have been noted in various reports.⁶ Originally, Fanconi¹¹ in 1927 reported cases occurring in three siblings. Sporadic as well as familial cases have been described. In most of the reported cases, hematologic abnormalities are initially detected between 4 and 12 years of age. An earlier onset has been observed in one infant at 13 months of age, and in two brothers symptoms of a blood disorder appeared at the ages of 19 and 20 years, respectively.¹² The anemia is normocytic and slightly microcytic, and leukopenia and thrombocytopenia coexist. As in patients with aplastic anemia, the bone marrow content varies from acellular to hypercellular.

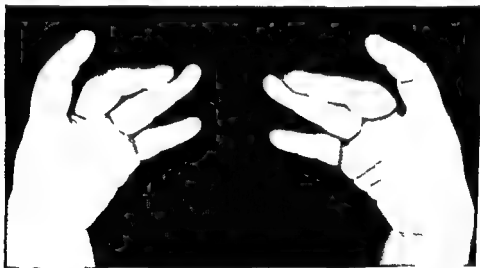


Fig. 11 Fanconi's syndrome showing bilateral absence of thumbs

The Fanconi syndrome appears to be transmitted by an autosomal recessive gene with variable penetrance. Treatment consists of supportive transfusions, steroid hormones, antibiotic therapy for infections, and splenectomy in selected cases. The severe bleeding tendency associated with thrombocytopenia is difficult to control, and in one patient this was accomplished by splenectomy.⁶ Patients with hypoplasia or maturation arrest have also been benefited by splenectomy.¹³ Indications for splenectomy are the same as in other types of aplastic anemia. Death usually results from hemorrhage into the brain or gastrointestinal

tract.⁶ Although the outlook is extremely grave for the majority of patients there are exceptional ones whose lives have been prolonged by modern therapy or whose survival is explained by minimal depression of the bone marrow.

Congenital Hypoplastic Anemia Without Associated Anomalies Estren and Dameshek¹ have reported several siblings in each of two families with generalized quantitative hypoplasia of all the elements of the bone marrow with the nucleated red cells in normal or elevated percentages. In one of their patients showing severe anemia with an increased number of reticulocytes and thrombocytopenia, splenectomy resulted in moderate clinical and hematologic improvement.

Acquired (Secondary) Aplastic Anemias

Depressed blood formation may result from various forms of injury to the bone marrow. The clinical features are identical with those of primary aplastic anemia but secondary anemia offers a better prognosis especially if the condition is diagnosed early and the offending agent is eliminated.

Anemia Due to Antimicrobial and Other Chemotherapeutic Agents The offending drugs and chemicals are of two types: those which regularly produce bone marrow depression when given in sufficient dosage (such as the compounds used in the treatment of leukemia and malignant lymphomas) and substances which are occasional bone marrow depressants.⁴ In the antileukemia group are nitrogen mustard, the folic acid antagonists, 6-mercaptopurine, busulfan (Myleran) and triethylenemelamine. Included in this category also are depressants are roentgen rays, radioactive phosphorus, radium and products of atomic fission or atomic bomb explosions.

In the category of the occasional offenders are those drugs and chemicals which produce aplastic anemia with the initial dosage or in a subsequent course. Triadione, Mesantoin and chloramphenicol are among the more common therapeutic agents which depress the bone marrow. Less common bone marrow depressants are sulfanilamide, sulfathiazole and sulfapyridine and among the antibiotics tetracycline, streptomycin and penicillin. Atabrine, neoarsphenamine among the organic arsenicals, gold and Butazolidin are also important etiologic agents but are of lesser importance in pediatric practice.

In the pathogenesis of aplastic anemia, chloramphenicol was probably the most frequent cause of aplastic anemia in the United States between 1949 and 1952.⁹ A sharp decrease in the total number of cases followed 1952 when the hazard was pointed out and precautions were advised.⁴⁰ Because of a recent increase in the number of patients in whom chloramphenicol was associated with the development of aplastic anemia, the cautious use of this drug has been re-emphasized.³⁴

Anemia Due to Industrial and Household Chemicals Benzol, benzene and benzene derivatives among the household and industrial chemicals have a high priority among the marrow depressants because of their widespread use as solvents and in the manufacture of drugs, dyes, shellac, paint removers and lacquers. Of importance also are DDT and other insecticides which are especially injurious in concentrated exposure within enclosed and poorly ventilated areas.

Anemia Due to Irradiation Roentgen rays and other forms of ionizing radiation are well known physical agents which cause marrow depression. Radiations are of two kinds. The first are those which penetrate the body tissues such as the roentgen rays (x rays) gamma rays and fast and slow neutrons. Gamma rays and neutrons are emitted from the explosion of atomic bombs and reach the blood-forming tissues rapidly. The second type are the alpha and beta particles which have a limited range of tissue penetration. They damage bone marrow directly when introduced into the body, as sometimes occurs in radium dial workers.³⁰

Except when given in tremendous amounts, irradiation damages the precursors of the hematopoietic system rather than the formed elements in the peripheral blood. The effect on the blood elements is related to their life span (the leukocytes having the shortest and the red cells the longest life span), the individual radiosensitivity of the precursors (the lymphocyte precursors being most sensitive) and the ability of hematopoietic tissue to regenerate (lymphatic tissue being most reactive). The leukocytes therefore show the first changes (namely, a decrease in the number of lymphocytes being most noticeable), followed by a reduction in the number of platelets and red cells. Granulocytopenia and thrombocytopenia lead to infection and hemorrhage. Neutropenia is usually among the first manifestations of chronic overexposure. Individual susceptibility, dosage and period of exposure account for the variation in blood alterations.

Miscellaneous Causes Untreated pernicious anemia, overwhelming infections and mechanical interference with erythropoiesis in the bone marrow may produce a blood picture of aplastic anemia. Anemias resulting from bone marrow invasion or replacement are the myelophthisic anemias due to bone marrow encroachment by foreign cells as occurs in patients with leukemia, lymphosarcoma, Hodgkin's disease, Niemann-Pick disease, Gaucher's disease, Letterer-Siwe disease, Albers-Schonberg disease (osteopetrosis) and myelofibrosis.

Features Common to Idiopathic and Acquired Types of Aplastic Anemia

Pathology The basic features of aplastic anemia are due to alterations of the bone marrow. During the course of the disease the bone marrow shows a progressive decrease in the total count so that the megakaryocytes eventually disappear, the myeloid elements and nucleated red cells are greatly reduced and the lymphocytes predominate in the smears. Patients with normal or hyperplastic bone marrows with a peripheral blood picture of aplastic anemia have been diagnosed as having maturation arrest or bone marrow block. Quantitative variations in the total nucleated cell count and distribution of the blood elements may be due to the fact that areas of relatively acellular marrow alternate irregularly with areas of normal or increased cellularity. When aplastic the normally red marrow appears yellow and fatty. The occasional increase in reticuloocytes is due to patchy areas of active blood formation in the bone marrow. The liver, spleen and lymph nodes are unaltered. Hemochromatosis may coexist with hemosiderosis due to iron deposition from multiple transfusions.³¹

Clinical Features The onset is insidious, rarely abrupt with anemia and

cutaneous hemorrhages as early features. It is soon noted that the thrombocytopenia and purpura are insufficient to account for the degree of anemia. The clinical features are closely dependent upon the blood changes: anorexia, weakness, easy fatigue, and dyspnea on exertion from anemia, and loss of blood. Epistaxis, bleeding from the gums, and gastrointestinal and renal tracts, menorrhagia, and retinal hemorrhage are prominent manifestations in later stages. Neutropenia is associated with sore throat and ulcerations of the mouth and pharynx. Fever due to secondary infections may be difficult to control with antibiotics. The spleen, liver, and lymph nodes are not enlarged.

Laboratory Findings. The red blood cell count fluctuates between 1.5 and 3 million per cubic millimeter; the hemoglobin drops proportionately. The reticulocytes number below 1 per cent and are sometimes absent. The red cells are normochromic and normocytic, but macrocytes are often numerous. The white cells tend to range between 1,500 and 4,000 per cubic millimeter, principally due to the lack of granulocytes. The platelets fall below 50,000 per cubic millimeter. Bone marrow aspiration discloses aplasia or hypoplasia. During the course of transfusion therapy, the serum iron already elevated in this disease becomes markedly increased, and the iron-binding protein becomes saturated, resulting in an absent latent iron-binding capacity.⁴

Differential Diagnosis. True aplastic anemia with an acellular bone marrow and the typical picture of profound anemia, leukopenia, neutropenia, and thrombocytopenia is a rare occurrence in infancy and childhood. The marrow reveals progressive hypoplasia, although scattered areas of normal composition may be noted in samples from different sites. The anemia develops insidiously so that diagnosis is difficult before pancytopenia is fully developed.

In differential diagnosis of aplastic anemia, the following causes of pancytopenia are to be considered:

- 1 Leukemia with aleukemic or subleukemic blood picture
- 2 Aplastic anemia
- 3 Bone marrow infiltration or replacement
 - A Malignant lymphomas
 - (1) Hodgkin's disease
 - (2) Lymphosarcoma
 - (3) Reticulum cell sarcoma
 - B Metastatic carcinoma
 - C Rare
 - (1) Osteopetrosis (marble bone disease)
 - (2) Myelofibrosis, myelosclerosis
- 4 Hypersplenic syndromes
 - A Banti's disease
 - B Lymphomas
 - C Gaucher's disease
 - D Niemann-Pick disease
 - E Letterer-Siwe disease
 - F Splenic pancytopenia
 - G Disseminated lupus erythematosus
- 5 Miscellaneous
 - A Megaloblastic anemias
 - B Overwhelming infections

Hemorrhage based on thrombocytopenia or a blood picture of chronic anemia may constitute the dominant features until the deficiencies of the other cellular elements appear. As already mentioned thrombocytopenic purpura which may be considered only in the course of the disease is associated with more than moderate anemia only as a result of blood loss. In the absence of overt hemorrhage the anemia must therefore be explained on another basis.

Acute lymphoblastic leukemia in the leukopenic stage may simulate aplastic anemia but microscopic study of aspirated marrow usually reveals the precise nature of the illness. With an acellular marrow the use of concentrated and volumetric bone marrow aspiration facilitates the differentiation between the two diseases.⁴⁸ A contracted or absent myeloid erythroid (nucleated cell) layer and normal or increased percentage of fat in aplastic anemia are contrasted with leukopenia from other causes in which the fatty layer is absent and the myeloid erythroid layer is variable.

Enlargement of the lymph nodes and spleen usually present in patients with acute leukemia is absent in those with aplastic anemia. In patients with splenic panhematopenia and secondary hypersplenism the spleen is predominantly implicated in the pathogenesis of pancytopenia and the bone marrow contains its full quota of each of the cellular elements. Myelofibrosis and neoplastic infiltration are also to be considered in the differential diagnosis.

Management. Comprehensive treatment consists of preventive measures, search for and elimination of the etiologic agent, transfusions and other supportive measures, stimulation of hemitopoiesis and consideration of splenectomy.

Preventive Measures. Because hypersensitivity is so variable and occurs so unexpectedly a selection of a drug which is potentially myelotoxic requires some deliberation and an attempt at supervision should be instituted if the drug is eventually to be used. These considerations apply particularly to patients with a history of allergic manifestations such as eczema, urticaria, chronic rhinitis, hay fever or asthma or who in the past have had skin rashes or constitutional reactions following exposure to a chemical or drug.

The extent to which precautionary measures are carried out depends therefore upon the known depressant effect of the drug, the benefits to be derived from its use and the inherent nature of the patient with respect to hypersensitivity reactions. Myelotoxic drugs are difficult to control because bone marrow hypoplasia can develop suddenly and because it is difficult to isolate the sensitive individual in whom treatment might be complicated by hemitopoietic depression. Even with drugs such as Trichone, Mesintom, Butizolidin and chloramphenicol which are known offenders the incidence of toxicity is extremely low in comparison with the large number of patients so treated. Nevertheless a hemoglobin test, white blood cell count and blood smear should be made periodically on patients receiving such drugs over prolonged periods and especially when treatment is intermittent. The exact intervals between these examinations cannot be specified. In the case of chloramphenicol weekly counts should be done initially and even more often when suspicious signs of toxicity appear. Fever, petechiae, purpura, epistaxis, oozing from the gums, mouth ulcerations, malaise, weakness, fatigue and skin rashes are important signs of bone marrow depression. In

the case of chloramphenicol withdrawal of the drug at the stage of granulopenia and anemia usually results in complete hematopoietic recovery.¹¹⁻¹⁴ In the marrow the signs of existing or impending bone marrow depression consist of a sudden shift to an immature level the presence of promyelocytes and myelocytes or marked reduction in the more mature elements and an increase in stem cells and lymphocytes.

Removal of the Causative Agent For obvious reasons recognition and elimination of the causative agent (such as a drug chemical insecticide or household chemical) are the first steps of treatment. Removal of the patient from further contact with the suspected toxic agent should be carried out immediately.

Transfusions and Other Supportive Measures Transfusions to combat anemia and provide platelets and ACTH and the adrenocortical hormones to stimulate hematopoiesis are administered as for pure red cell anemia. Infections are often difficult to control even with the liberal use of wide spectrum antibiotics. Reliance is placed on penicillin streptomycin and tetracycline until more specific information is obtained about the invading organism from blood cultures and examination of other accessible infected sites. In the event of neutropenia antibiotics are given if there are other clinical manifestations of active disease. The prophylactic use of antibiotics is hazardous because of possible bacterial resistance.

Children with proved aplastic anemia have recently been treated by Shahidi and Diamond¹⁵ with testosterone combined with corticosteroids over prolonged periods with resulting clinical and hematologic improvement. During the remission the hemoglobin reached normal levels the number of reticulocytes increased and the bone marrow was converted from a markedly hypocellular and fatty marrow to a hypercellular one with erythroid hyperplasia. More extensive experience is needed with this promising approach.

Testosterone was used as testosterone propionate or methyltestosterone in the form of 10 mg buccal Linguets in a daily dosage of 1 or 2 mg per kilogram of body weight. Corticosteroids were given in addition as triamcinolone in a dosage ranging from 8 to 12 mg daily. Testosterone is also given intramuscularly for those who cannot manage the Linguets. Although the dosage is still empirical the drug has been given in doses of 100 mg once or twice a week.

Control of Hemorrhage Thrombocytopenia almost invariably leads to excessive bleeding which is difficult to control and is often responsible for a fatal outcome. Transfusions of fresh whole blood and platelet rich plasma (obtained after sedimentation or centrifugation) collected in plastic or siliconized equipment supplies intact platelets or platelet material for brief periods. Reliance is placed on ACTH and adrenocortical steroids to control hemostasis. This is accomplished more by increase of capillary resistance than by elevation of the number of platelets.¹⁶

Bleeding in aplastic anemia including the Fanconi type is occasionally amenable to splenectomy. In two girls 9 and 12 years of age diminution of generalized bleeding and severe menorrhagia respectively resulted from splenectomy when all other available measures for controlling hemostasis failed. In a 15 year old girl with the Fanconi type of aplastic anemia splenectomy was

performed because of severe thrombocytopenia and bleeding. At 20 years of age she was no longer anemic and the bleeding tendency had subsided but thrombocytopenia and leukopenia persisted.⁶

Marrow Transplantation Efforts to suppress or abolish the immune response to permit marrow transplantation have not as yet proved successful.⁵³

Splenectomy The rationale for splenectomy has been reviewed in connection with pure red cell anemia. Recently this procedure has been recommended more freely in patients with unexplained refractory anemia especially when any improvement in bone marrow function could be predicted. In one series six of twelve patients with hypoplastic disease of the bone marrow¹ in whom the indications for splenectomy were the presence of severe thrombocytopenic purpura, anemia, and granulocytopenia leading to increased susceptibility to infection improved. Five of the twelve patients ranged in age from 2 to 9 years and of these two showed hematologic improvement but in only one was it sustained. In another series⁴⁰ four of fifteen splenectomized patients have shown partial or complete recovery. It appears that splenectomy should be reserved not only for those patients in whom a hemolytic component can be demonstrated but also for those in whom an inhibiting effect of an abnormal spleen on the marrow can be postulated. The criteria for patients with completely aplastic marrows still await formulation.

Miscellaneous Treatment The administration of iron is potentially harmful in patients with refractory anemias because of its increased absorption from the gastrointestinal canal supplementing the already excessive iron stores within the tissues from transfusions. The use of cobalt has had many advocates with sporadic reports of beneficial effects in pure red cell anemia and other refractory anemias. Cobalt has been shown to influence the production of the erythropoietic stimulating factor.¹⁹ Its possible side effects such as anorexia, nausea, and thyroid enlargement preclude its widespread use except under controlled conditions.³ Favorable results with cobalt however have been reported in several adults with the acquired erythrocytic hypoplasia.^{14,41} In our experience the results with cobalt in congenital pure red cell anemia have been uniformly disappointing.

Each of the following vitamin B factors in suggested dosage has been given to patients with pure red cell anemia and other members of the hypoplastic aplastic group of anemias: vitamin B₁₂ 1 000 micrograms intramuscularly three times weekly; riboflavin 10 mg three times daily; nicotinamide 50 mg daily; and folic acid 5 mg three times daily. In the doses mentioned beneficial effects were obtained with riboflavin in the treatment of pure red cell anemia¹ and nicotinamide in hypoplastic anemia after the administration of chloramphenicol.⁴⁰ Although many of these substances are known to function in specific phases of erythropoiesis their value in patients with refractory anemia is still debatable. Occasionally they appear to stimulate red cell formation particularly after splenectomy.

Course and Prognosis The outcome of idiopathic aplastic anemia in childhood is almost uniformly fatal. The acute course of weeks and months is marked by intractable hemorrhage and infection. The prognosis is better if the cause is

known and in patients with cellular rather than acellular marrows. When a drug such as chloramphenicol is discontinued, hematologic improvement is gradual, the first sign of improvement being a decreased transfusion requirement followed by a rise in the number of neutrophils and platelets. The platelets are the last of the blood elements to become normal in recovered patients.⁴⁰ Cerebral hemorrhage and septicemia are the common causes of death.

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The Hemolytic Anemias

Definition—General Considerations of Pathogenesis A hemolytic anemia may be defined as one due to excessive blood destruction. A large number of blood disorders of varied etiology and clinical and hematologic course are included in the group of hemolytic anemias which have in common the central feature of a red cell with a reduced life span.

As has been pointed out, the presence or absence of anemia is dependent upon the ability of the bone marrow to respond to varying degrees of blood destruction. Abnormal degrees of hemolysis therefore need not result in anemia if the bone marrow is capable of regenerating adequate amounts of hemoglobin and red blood cells. Bone marrow capacity has been estimated at 6 to 8 times the normal so that when the average red cell survival is reduced to 15 to 20 days instead of the normal 120 days the limit of compensation has reached its possible maximum. This situation represents a completely compensated hemolytic disease without apparent evidence of anemia. However, if the rate of destruction exceeds the ability of the body to increase the rate of red cell production anemia will result. This is termed decompensated hemolytic disease. A new equilibrium is eventually reestablished when these forces are equalized at lower hemoglobin levels. The particular concentration of hemoglobin obtained is therefore determined both by the extent of the hemolytic defect present and by the particular capacity of the bone marrow to respond with an increase in the production of erythrocytes.

Classification Hemolytic anemias may be classified as congenital (hereditary) and acquired—the former related to an intracorpuseular (intrinsic) abnormality and the latter related to an extracorpuseular (extrinsic) mechanism and due to the action of external agents on structurally normal red cells. A combination of factors may be present in the same patient as in patients with Cooley's anemia in whom the abnormal cells have a shortened life span within the circulation and transfused normal donor cells are destroyed by an extracorpuseular mechanism residing in the spleen (hypersplenism).

1 Intracorpuseular or intrinsic al normality

- A Hereditary (congenital) hemolytic anemia
 - (1) Hereditary spherocytosis
 - (2) Thalassemia

- (3) Sickle cell disease
- (4) Hereditary hemoglobinopathies including combinations of thalassemia and sickle cell disease
- (5) Hereditary nonspherocytic hemolytic anemias
- (6) Hereditary elliptocytosis with hemolytic anemia
- (7) Hemolytic anemia due to enzyme deficiency apparent after administration of drugs and other agents primaquine naphthylene fava bean (favism) etc

II Nonhereditary hemolytic anemia

- (1) Paroxysmal nocturnal hemoglobinuria

III Extracorporeal or intrinsic mechanism

A Acquired hemolytic anemia

- (1) Due to antibodies
 - (a) Autoimmune antibodies
 - (1) Idiopathic acquired hemolytic anemia
 - (b) Isoagglutinins
 - (1) Erythroblastosis mismatched transfusion (blood or plasma)
- (2) Symptomatic hemolytic anemias
 - (a) With Hodgkin's disease lymphosarcoma renal disease lupus erythematosus thrombotic thrombocytopenic purpura etc
- (3) Paroxysmal cold hemoglobinuria
- (4) Acute acquired hemolytic anemia (Lederer's anemia)
- (5) March hemoglobinuria
- (6) Miscellaneous causes
 - (a) Infections
 - (b) Chemical and physical agents
 - (c) Vegetable substances
 - (d) Drug sensitivity
 - (e) Severe burns

It is reasonable to assume then that in any patient in whom the rate of destruction of erythrocytes less than 6 to 8 times the normal no anemia need occur because of the inherent compensatory capacity of the bone marrow. An exception to this principle was noted in a series of pediatric patients with minimal clinical manifestations of hereditary spherocytosis in whom anemia was present in terms of a reduced total volume of red cells (red cell mass) despite a normal concentration of hemoglobin in the peripheral blood.⁴⁷ The capacity of the bone marrow was inadequate to compensate for rates of erythrocyte destruction of a magnitude less than 6 times the normal. The normal peripheral hemoglobin is explained by the contracted plasma component of the circulating blood. The studies of Crosby and Akeroyd⁴⁸ therefore need elaboration to determine the full range of the bone marrow's capacity under the stress of varying degrees of blood destruction.

Principal Features of Increased Hemolysis The varied clinical and hematologic manifestations of a hemolytic process stem from the release of products from red cell destruction and their disposal and the compensatory activity of the bone marrow. Many of these changes are observed in the urine, stool, and blood and are subject to quantitative measurement. The evidences of increased red cell destruction to be enumerated include anemia, structural abnormalities of the red cells, and increased pigment output.

Hemoglobinemia and Hemoglobinuria The concentration of hemoglobin nor

mally found in the plasma is less than 5 mg per 100 ml and results from red cell destruction taking place within the cells of the reticuloendothelial tissues. In the event of severe intravascular hemolysis the concentration of plasma hemoglobin usually exceeds the renal threshold of 100 to 150 mg per 100 ml and the pigment appears in the urine.⁷ Hemoglobinuria occurs with marked hemoglobinemia when the rate of destruction is exceedingly rapid and severe indicating that hemolysis is taking place in the circulating blood. Depending upon the amounts of pigment present the urine is pink, red, brown or almost black. Hemoglobinuria can be detected by benzidine and guaiac reactions. In patients with marked hemoglobinemia the plasma is red or brown. The absence of hemoglobinemia in patients with hemolytic disease indicates that extravascular hemolysis is principally involved. Hemoglobinemia is absent in patients with hereditary spherocytosis and hereditary nonspherocytic hemolytic anemia;⁸ mild hemoglobinemia is present in those with sickle cell anemia and Cooley's anemia. Severe grades of hemoglobinemia occur in patients with acute acquired hemolytic anemia such as Lederer's anemia.

In patients with marked hemoglobinemia iron containing brownish granules of hemosiderin appear in the urine. Treating the urine with acidified potassium ferrocyanide reveals the presence of the Prussian blue hemosiderin.⁹ The extent of hemosiderin excretion varies with the concentration of plasma hemoglobin. Hemosiderinuria is a characteristic finding in patients with nocturnal paroxysmal hemoglobinuria in whom hemoglobinemia is usually present.

Haptoglobin in Relation to Hemoglobinemia When intravascular hemolysis occurs the free hemoglobin in the plasma combines with a specific serum protein haptoglobin (Hp) comparable to the union of copper with ceruloplasmin and of iron with siderophilin or transferrin. Haptoglobin is an alpha mucoprotein, 1 molecule of which is capable of binding 2 molecules of hemoglobin.¹⁰ In adults the haptoglobins are present in amounts sufficient to bind up to 150 mg of hemoglobin per 100 ml of serum.¹¹ The haptoglobin-hemoglobin complex is removed from the plasma by the reticuloendothelial system where the hemoglobin is degraded into bilirubin.

The large molecular weight of the haptoglobin-hemoglobin complex (310,000) prevents its passage into the urine. Hemoglobinuria does not occur unless the amount of hemoglobin liberated intravascularly exceeds the binding power of the haptoglobin and the reabsorption capacity of the tubules. Unbound free hemoglobin is also oxidized into methemoglobin and methemalbumin which are also eliminated into the urine. It has been demonstrated by starch electrophoresis that normal serum contains three haptoglobins. These are genetically controlled^{11a, 11b} and one or more may be present in the serum of a single person. The concentration of haptoglobin is increased in patients with infections and malignancy and in those being given steroid therapy and it is decreased or even absent in patients with increased hemolysis.^{12a} A reduced haptoglobin and therefore decreased hemoglobin binding may account for increased methemalbumin formation and in certain cases for increased hemoglobinuria.

Haptoglobins cannot be demonstrated in cord blood or in the early neonatal period. They appear initially at the end of the first or second week of life.^{1 11b}

Methemalbumin and Methemalbuminemia Methemalbumin is formed in the plasma by the union of hematin with albumin when excessive amounts of hemo- globin are released in the course of severe red cell destruction.¹ Like haptoglobin it serves as a means of transporting and neutralizing products of hemolysis in this case hematin in the blood stream. When hemoglobinemia is intense the spectroscopic picture of methemalbumin is distinct. In lesser amounts it can also be differentiated from methemoglobin and allied compounds by the effect of chemical reagents on their "a" bands. The large sized molecule prevents its passage through the kidney and its presence in the urine.

Hyperbilirubinemia and Jaundice The extent of bilirubinemia depends upon the degree of hemolysis and the capacity of the liver to remove the pigment from the plasma and excrete it in the bile. In the infant in the first week of life the excretion of bile is retarded by the inability of the immature liver to conjugate indirect bilirubin because of enzyme deficiency. In the older patient the functional capacity of the liver may be impaired by the coexisting anemia. Usually the liver is able to excrete increased amounts of bilirubin so that the degree of hyperbilirubinemia may not reflect the amount of blood destruction. In patients with hemolytic anemia the plasma bilirubin ranges from 1 to 3 mg. per 100 ml. and is occasionally normal so that the absence of jaundice does not necessarily eliminate a diagnosis of hemolytic anemia.

Urobilinogen Excretion The liver is able to excrete large amounts of bilirubin unless its function is impaired by severe anemia and anoxemia associated with the hemolytic process. Only small quantities of urobilinogen are excreted by the kidneys. Increased blood destruction is associated with excessive urobilinogen excretion in the feces and frequently in the urine. With the limitations already cited elevated fecal urobilinogen may be considered as evidence of hemolytic disease but the amount present in the urine is not a reliable measure of this process.

Spherocytosis In addition to spherocytes which characterize the blood picture of hereditary spherocytosis acquired types appear in hemolytic disease which are morphologically identical with the former. The presence of spherocytes may either indicate the production of intrinsically defective erythrocytes or reflect damage to normal cells by extrinsic factors. Spherocytes appear in patients with ABO erythroblastosis fetalis hemolytic anemias due to drug sensitivity and idiopathic acquired hemolytic anemia. In the latter spherocytes are sometimes more numerous than in patients with hereditary spherocytosis. The acquired type of spherocyte represents a normal red cell whose surface has been damaged causing irreversible contraction with an increase in osmotic and mechanical fragility. Spherocytes occur in so many hemolytic anemias of varied etiology that they are not diagnostic of any single one of them.

Schistocytes Red cell fragments as well as shrunken and distorted red cells seldom seen in normal blood are present in appreciable numbers in patients with hemolytic disease. Red cell fragmentation has been described following severe burns⁶³ and exposure to chemicals.

Erythrophagocytosis Erythrocytes ingested by polymorphonuclear neutrophils and monocytes are rarely found in normal blood smears but appear in those of

patients with hemolytic anemias such as erythroblastosis acquired hemolytic anemia leukemia chemical poisoning and anemias associated with infections

Heinz Bodies Heinz bodies are red cells damaged by toxic substances leading to the appearance of intracorpuseular particles and larger rounder bodies. A variety of drugs such as phenylhydrazine potassium chloride naphthalene primaquine and related compounds have been implicated in their formation. They are best observed in wet unstained preparations or with the brilliant cresyl blue technique for reticulocyte staining.

Evidence of Increased Marrow Activity In achieving equilibrium red cell destruction is accompanied by graded increases of red cell production. The latter is marked by a compensatory hyperplasia of the bone marrow resulting in proliferation of all types of nucleated red cells ranging from the early proerythroblasts to the most mature normoblast. Also included in the blood picture of hemolysis are reticulocytosis normoblastemia macrocytosis polychromatophilia polymorphonuclear leukocytosis and increased numbers of platelets.

Reticulocytosis The appearance of nucleated red cells and reticulocytes in the blood smear indicates the premature delivery of red cells into the circulation. Reticulocytes usually range between 0.5 and 2 per cent in the period beyond early infancy. They are increased in patients with hemolytic anemia and hereditary spherocytosis and in those with acquired hemolytic anemia they may number approximately 20 to 50 per cent. An increase of reticulocytes does not always serve as an accurate index of red cell production since bone marrow hyperplasia may be associated with only slight increases in reticulocytes as seen in patients with sickle cell anemia and severe Cooley's anemia. Reticulocytopenia has been noted during a hemolytic (not aplastic) crisis in patients with acute immune hemolytic anemia. This is explained by the suppressive action of the autoimmune mechanism on the proliferating erythroid cells. In the aplastic crisis reticulocytes disappear from the peripheral blood and nucleated cells from the bone marrow. Thrombocytopenia and leukopenia are accompanying features. The aplastic crisis is observed more frequently than the better known hemolytic crisis and is particularly common in patients with hereditary spherocytosis sickle cell anemia and Cooley's anemia.

Normoblastemia The more anemic the patient and the higher the reticulocyte count the more likely are normoblasts to appear in the peripheral blood. They are particularly noticeable in patients with erythroblastosis fetalis and severe autoimmune hemolytic anemia and after splenectomy in those with Cooley's anemia.

Siderocytes Siderocytes are erythrocytes containing one or more granules of ferric iron which give a positive Prussian blue reaction for iron. They are increased in the peripheral blood of patients after splenectomy⁴ as well as in patients with certain types of hemolytic anemia. These include erythroblastosis fetalis hereditary spherocytosis and autoimmune hemolytic anemia. Siderocytes appearing in the peripheral blood in patients with hemochromatosis reflect excessive red cell storage of iron.^{5a}

Sideroblasts Sideroblasts are the precursors of siderocytes in the peripheral blood. Siderotic granules appear in the normoblasts of patients with normal bone

marrow. They are diminished in patients with iron deficiency states⁷ and are exceptionally abundant in those with hemolytic disorders: severe Cooley's anemia, megaloblastic anemia, and lead poisoning.

Macrocytosis. An increased number of reticulocytes in the peripheral blood and micronormoblasts in the bone marrow account for a macrocytic blood picture in patients with hemolytic disease.

Increased Erythrocyte Protoporphyrin and Coproporphyrin. The heme portion of hemoglobin is a ferrous protoporphyrin for whose synthesis porphyrin precursors and iron are required. The porphyrins are pigments whose structural unit consists of four pyrrole rings linked by four methene bridges. They differ depending upon the nature of the side chains attached to the eight free corners of the four pyrrole nuclei. Thus hemoglobin, myohemoglobin, cytochrome and catalase represent porphyrin protein compounds. Most porphyrins exist in the form of metal complexes (metalloporphyrin)—for example, hemoglobin and chlorophyll, in which porphyrins are combined with iron and magnesium respectively. Coproporphyrin, uroporphyrin, and protoporphyrin are the most common porphyrins occurring naturally.

Porphyrinuria describes a condition in which there is increased urinary excretion of coproporphyrin as in patients with liver disease, hemolytic anemia, and lead poisoning. Porphyrins are rare metabolic disorders in which abnormal amounts and kinds of porphyrins are excreted in the urine and feces.⁸

Congenital porphyria is a very rare metabolic disease inherited as a recessive character whose clinical features appear in infancy and childhood. It is characterized by sensitivity of the skin to sunlight resulting in vesicles and bullae (hydra vesiculae), hirsutism, red urine, red or purplish brown staining of the teeth, an enlarged spleen, and a hemolytic anemia. Scarring occurs on the crusts of the vesiculobullous lesions and in other exposed areas.

The biochemical defect is ascribed to the failure to convert uroporphyrin formed in excessive amounts to coproporphyrin. Uroporphyrin and coproporphyrin are the predominant porphyrins excreted. The continuous deposition of uroporphyrin in tissues, dentine, and the skeleton accounts for the hypersensitivity and pigmentation of bones and teeth.

Treatment consists of shielding the patient against sunlight. Splenectomy, which is indicated with increased blood destruction, reduces the concentration of uroporphyrin and coproporphyrin in red cells, plasma, urine, and feces. The plasma porphyrins prior to splenectomy are probably derived from the destruction of circulating red cells containing these substances.

Human erythrocytes contain free coproporphyrin (up to 2 micrograms per 100 ml of red blood cells) as well as free protoporphyrin (normal 15 to 60 micrograms per 100 ml of red blood cells). In patients with iron deficiency anemia and lead poisoning marked increases of protoporphyrin occur. Moderate increases are found in patients with hemolytic anemias and regenerative anemias. In persons with hemolytic anemias there is a relatively greater increase in the erythrocyte coproporphyrins than in protoporphyrins. The presence of large amounts of uroporphyrin and coproporphyrin in the normoblasts of the bone marrow has suggested that these substances are formed in the marrow. Erythropoietic porphyria, therefore, has been applied as an alternative designation to congenital porphyria.

Additional Tests for Detecting Abnormal Hemolysis. The vulnerability of the red cells to hemolysis may be determined by a few well defined tests:

Osmotic Fragility Test The resistance of the red corpuscles to varying dilutions of hypotonic sodium chloride solution constitutes an important laboratory procedure in the diagnosis of the anemias and is especially useful in the differentiation of the hemolytic group. The response of blood cells in the fragility test does not necessarily reflect their reaction within the circulating blood where conditions of isotonicity prevail. It constitutes however a useful gross index of the relative thickness of the major number of red cells in the sample of blood to be tested. If the red cell envelope is small in relation to its volume as in the thick red cells of patients with hereditary spherocytosis the resistance is decreased. Increased resistance to hemolysis is noted when the bulk of red blood cells possess relatively large surfaces in comparison with their substance as occurs in the thin red cells of patients with thalassemia major and iron deficiency anemia.

Increased fragility of the red cells in hypotonic solutions of sodium chloride as compared with the fragility of normal blood occurs not only in persons with hereditary spherocytosis but occasionally in those with acute hemolytic anemia associated with infection and ingestion of drugs. Decreased fragility with complete hemolysis in solutions below 0.3 per cent sodium chloride characterizes the blood of patients with sickle cell anemia, mild and severe forms of thalassemia and iron deficiency.

METHODS Osmotic resistance may be measured by placing small samples of blood in solutions of progressively diminishing concentrations of sodium chloride. After the solutions have been allowed to stand for a prescribed time the points of minimal and maximal hemolysis are noted. Hemolysis normally begins in 0.42 per cent solution and is complete in 0.33 to 0.30 per cent.

An alternative and more precise method is the photoelectric determination of the degree of hemolysis in which the differences in the degree of hemolysis occurring in successive dilutions of hypotonic salt solution are plotted.¹¹

The red cells in these solutions absorb water and grow progressively larger and more rounded until their membrane is stretched to the point of rupture when hemoglobin is liberated. Red cells already rounded as those in patients with autoimmune acquired hemolytic anemia and hereditary spherocytosis begin to hemolyze at more concentrated levels than normal. A thinner than normal red cell absorbs more water before rupture of the red cell membrane occurs.

OSMOTIC FRAGILITY AFTER INCUBATION The more rapid increase in the osmotic fragility of red cells after incubation for twenty-four hours at 37° C. is useful in detecting the asymptomatic subject with hereditary spherocytosis whose red cells show a normal fragility at room temperature. This method is especially applicable to the parent of a child with overt evidence of hereditary spherocytosis in whom a hereditary pattern could not otherwise be elicited.¹²

Screening Fragility Test Using Finger Tip Blood A screening test which has been shown to offer information of a decisive diagnostic character employs blood from the finger and three dilutions of hypotonic solution of sodium chloride—0.375 per cent, 0.30 per cent and 0.325 per cent. The method as outlined here is not meant to replace the routine fragility test but serves as a gross method in screening patients suspected of having thalassemia minor or major. Since blood from the finger is used, controls are readily available and in normal persons

the degree of hemolysis in the three tubes is as follows: with 0.375 per cent solution of sodium chloride 3 plus, with 0.35 per cent 3 or 4 plus, and with 0.325 per cent 4 plus.

Two or three drops of blood from the finger depending upon the severity of the anemia are obtained by deep puncture and then added to each of the three tubes each containing 2 ml. of solution of the prescribed concentrations of sodium chloride. After thorough mixing of the blood and solution the tubes are allowed to stand in a refrigerator until sedimentation has occurred. The degree of hemolysis and the residue of unhemolyzed red cells are compared with the same factors in the blood of a normal control treated in the same manner. If any clotting occurs the tube is centrifuged at low speed for a few minutes and the degree of hemolysis in the supernatant fluid is compared with that found in the corresponding tube of the control. Additional tubes with 0.45 and 0.425 per cent of sodium chloride will detect the increased osmotic fragility of the blood from patients with hereditary spherocytosis. These simplified tests provide prompt information and are designed for screening.

Autohemolysis Test Normal defibrinated blood incubated under sterile conditions at 37° C. shows minimal lysis at the end of forty-eight hours (not more than 5 per cent). In blood from patients with hereditary spherocytosis and pyrocytic nocturnal hemoglobinuria the rate of autohemolysis is greatly accelerated. In general the rates of hemolysis parallel the degree of increased fragility before incubation.⁹ Selwyn and Dacie¹¹¹ reported that spontaneous autohemolysis in patients with hereditary spherocytosis is not due to progressive swelling of the cells but to a defective cell membrane which undergoes degenerative changes more rapidly than normal. They also found that autohemolysis of the red cells of patients with hereditary spherocytosis was markedly retarded when appreciable amounts of glucose were added to the blood before incubation. Abnormally rapid autohemolysis is also found in patients with other types of spherocytosis and nonspherocytic hemolytic anemia.¹

Investigation of Red Cell Survival in Patients With Hemolytic Disorders The use of radioactive sodium chromate for labeling red cells provides a convenient method for estimating red cell survival. Since the isotope (Cr^{51}) remains attached to the erythrocyte for considerable periods of time the life span of the patient's red cells can be determined within his own circulation or in a normal recipient. By thus tagging the patient's red cells with Cr^{51} it is possible not only to measure the rate of hemolysis but also to demonstrate the principal site at which this occurs. This is accomplished by reinjecting the patient's own Cr^{51} labeled red cells and measuring the selective uptake of radioactivity over the heart, liver, and spleen. A shortened survival of labeled red cells in the circulation and a much greater radioactive count over the spleen as compared with the liver suggest that the patient would benefit from splenectomy.⁶ Not only autogenous cells but also the shortened survival of normal donor cells in patients with the refractory hemolytic anemias such as severe Cooley's anemia may be accompanied by a progressive increase of radioactivity over the spleen. This finding incriminates the spleen as the site of excessive hemolysis for donor cells and serves as one of the criteria for splenectomy.

Aplastic Crisis in Hemolytic Anemia In the study of the course of hereditary spherocytosis Owen¹ observed that the crisis which characterizes this disease was not associated with an increase in hemolysis but with a sudden cessation of erythropoiesis, reticulocytopenia, increased prothrombin rather than prothrombin time, a de-

creased concentration of serum bilirubin and varying degrees of leukopenia and thrombocytopenia. Instead of an increased hyperplasia the bone marrow was depressed especially with respect to erythropoiesis. Spontaneous recovery was associated with the rapid regeneration of erythropoietic tissue, marked reticulocytosis, leukocytosis, and an increase in platelets. The crises are self limited, lasting one to two weeks. Owren's conclusion that the crises in patients with hereditary spherocytosis are aplastic rather than hemolytic has been amply confirmed and observed in patients with other hemolytic anemias. There is no uniform causative factor for the crisis, although infection is regarded as the most common agent.

Aplastic crises have been described in children suffering from sickle cell anemia^{18, 19}, acquired hemolytic anemia²⁰, and paroxysmal nocturnal hemoglobinuria. Transient aplastic or hypoplastic states are occasionally observed in patients with severe Cooley's anemia during infection and often without a precipitating illness.²⁰ An infectious etiology explains the occurrence of aplastic crises within a brief period in several members of families with sickle cell anemia.⁹ A transitory disappearance of normoblasts from the bone marrow has been described in allergic children as a result of infection due to sensitivity to a drug.⁷

It is obvious that the severity of the anemia during and following the aplastic crisis is correlated with the life span of the red cells characterizing the anemia. The degree of anemia would be less marked in a person whose red cells had a normal life span of 120 days as compared with the short life span of the spherocyte or sickle cell. In the patient with sickle cell anemia an acute cessation of red cell production for a few days can result in a serious drop in hemoglobin and red cells. In a patient with infectious mononucleosis in whom the life span of the red cell is normal, transitory erythroblastopenia was followed by minimal anemia.²¹ Such minor degrees of anemia in previously nonanemic persons are readily remedied on the resumption of erythropoiesis.

Less commonly a true hemolytic crisis is encountered in patients with sickle cell anemia as well as in those with the more common aplastic crisis. It is characterized by a severe pain in the back, abdomen, and extremities, with a significant drop in erythrocytes and hemoglobin level, and by an increase in serum bilirubin and reticulocytes. In this situation there is a dramatic fall in the circulating red cell volume accompanied by hyperplastic bone marrow.¹⁰

CONGENITAL HEMOLYTIC SYNDROMES

The hereditary hemolytic syndromes, many of which exhibit an intrinsic abnormality of the red cell hemoglobin or both, include a large variety of disorders. They embrace hereditary spherocytosis, hereditary elliptocytosis, hereditary nonspherocytic hemolytic anemia, Cooley's anemia, sickle cell anemia, and many of the hereditary drug induced hemolytic anemias. These erythrocytic abnormalities differ from each other morphologically, and when this is not obvious they are distinguished by specific laboratory tests. The genetic patterns also serve as diagnostic aids. Paroxysmal nocturnal hemoglobinuria, due to an inherent

red blood cell defect is neither congenital nor hereditary. Sickle cell disease and thalassemia will be considered with the hereditary hemoglobinopathies in Chapter 16.

Hereditary Spherocytosis (Congenital Hemolytic Jaundice, Congenital Hemolytic Anemia, Spherocytic Anemia, Chronic Acholuric Jaundice, Chronic Familial Jaundice)

The designation hereditary spherocytosis emphasizes two important elements of the disease without excluding cases in which anemia and jaundice are absent. Hereditary spherocytosis is one of the four chief congenital hemolytic syndromes which exhibit a hereditary abnormality of the red cell, the others being hereditary elliptocytosis, hereditary nonspherocytic hemolytic anemia, Cooley's anemia, and sickle cell anemia.

Definition. Hereditary spherocytosis is a genetically determined chronic hemolytic disease characterized by spherocytes, increased osmotic fragility of the red cells, and frequently splenomegaly.

Inheritance and Race. The disease is found chiefly among Caucasians and rarely among Negroes.⁹⁻¹³ The defect is transmitted by either parent as a mendelian dominant so that males and females are equally affected. Discrepancies in the hereditary transmission which are difficult to explain are observed. In one series only 24 per cent of the offspring of families with an affected parent showed spherocytosis instead of the expected 50 per cent.¹⁴ Affected children have also been observed both of whose parents possessed no clinical or hematologic evidence of this disease. Such exceptions are explained on the basis of doubtful paternity, mutation, or incomplete expressivity of the gene for hereditary spherocytosis.⁹ Occasionally one of the apparently normal parents will reveal the red cell defect of spherocytosis by increased osmotic fragility or autohemolysis in excess of the normal after incubation of a blood sample at 37° C for twenty-four to forty-eight hours.

Etiology and Pathogenesis. The basic defect in patients with hereditary spherocytosis consists of the production of spherocytes which, because of their shape, are trapped in the spleen and are destroyed at a more rapid rate than normal. The pathogenesis of the abnormal shape of the spherocyte has been investigated with respect to its metabolic derangements and the effect of stasis within the spleen. The spheroidal configuration is not present in the corresponding nucleated red cells in the bone marrow but appears in the reticulocytes and is established thereafter.¹⁵ Less often a definite but slight reduction in size has been detected in the late normoblast.²⁵ Once having gained maturity in the circulation, the non-nucleated red cell is smaller and thicker than the corresponding normal red cell.

Extensive studies of various metabolic alterations in the spherocytes have been conducted. Disturbances in carbohydrate metabolism in these cells leading to slow regeneration of high energy phosphate bonds have been demonstrated, but the relationship of these findings to the shape and susceptibility to hemolysis of spherocytes is not yet clear.^{9, 104, 105}

It is generally accepted that the globular shape of the red cells accelerates their removal from the circulation and their sequestration in the splenic pulp.

where they undergo lysis. This process is not dependent upon specific abnormalities of the patient's spleen since it takes place as readily in the spleen of normal persons as in that of those suffering from diseases other than spherocytosis. The abnormal thickness of spherical cells does not permit them to escape readily from the pulp into the venous sinuses.¹ The stagnation of spherocytes in the spleen is apparent in the engorgement of the pulp with red cells but the venous sinuses are for the most part inconspicuous and contain few red cells.^{1,2} Increasing evidence has confirmed the hypothesis that many of the erythrocytes having been trapped in the spleen undergo lysis and others are so conditioned that they are incapable of prolonged survival in the circulation after release from the spleen.^{4,5} Additional evidence that the spherocytes are primarily involved in the pathogenesis is the fact that removal of the spleen relieves the anemia but the spherical red cells persist and their properties of increased osmotic and mechanical fragility remain unaltered.

Clinical Features The clinical manifestations are extremely variable particularly with regard to the time of onset and intensity of symptoms. In individual patients and among members of the same family they vary widely at different times in the course of the disease. Jaundice symptoms referable to anemia and splenomegaly constitute the most common clinical features of the disease. In some infants hereditary spherocytosis may be evident at birth whereas in others symptoms may not appear until late in adult life.

Jaundice is unusual in infancy and childhood except during the newborn period when it may simulate erythroblastosis with the same susceptibility to kernicterus in the event of hyperbilirubinemia. In the older child an icteric tint is noticeable which becomes more pronounced in the young adult and affected parent. Jaundice is accompanied by a highly pigmented urine and stool. The spleen is nearly always enlarged. In the young infant it may be palpable or may not be felt at all. Pallor with or without anemia and a sallow complexion are common. Lassitude, fatigability, anorexia, fever and abdominal pain may call attention to the disease. Crises are ushered in by fever, extreme pallor, shortness of breath, nausea, vomiting and extreme weakness. They are now known to be aplastic rather than hemolytic as formerly assumed and are associated with cessation of blood formation. Crises may occur in several members of the same family almost simultaneously.

Gallstones occur commonly in older persons with an incidence as high as 85 per cent of cases;^{1,2} they are uncommon in young children but have been reported in a child 3 years old. They usually increase in frequency after the age of 10 years.¹ Gallstone colic or biliary obstruction may constitute the first indication of existing hereditary spherocytosis. A history of cholelithiasis in a parent justifies examination for spherocytosis in the offspring, who is pale, easily fatigued and without appetite. The presence of gallstones in patients with hereditary spherocytosis depends upon the activity and duration of the hemolytic process.

Leg ulcers occur infrequently and are extremely rare in childhood. Their causation is not so readily explained as in patients with sickle cell anemia. Cardiac murmurs, especially in young children, may be so intense as to simulate

congenital heart disease and lead to recognition of the underlying disease. Their elimination by transfusion confirms a hemic origin. Roentgenologic changes are similar to those occurring in patients with other congenital hemolytic syndromes but less in degree and incidence. They most commonly consist of thickening and striation of the frontal and parietal bones of the skull.¹⁵

Growth. Among patients with hereditary spherocytosis who exhibit little in the way of symptoms but have a reduced volume of erythrocytes, growth has been normal. Except during transient periods of marrow hypoplasia (aplastic crisis), values for hemoglobin concentration ranged from 9 to 14 gm per 100 ml. There was no deviation from the normal growth curve in any patient during the period of the expected preadolescent acceleration of growth. It is during this period that patients with other chronic anemias such as thalassemia major receiving transfusion therapy fail to keep pace with rates of growth observed in normal children. Thus adults with congenital spherocytosis who are relatively asymptomatic except during periods of crisis are of normal height. In contrast to these patients, children who are moderately or severely anemic and require repeated transfusions show impairment in both growth and weight. In such patients it is undoubtedly wise to advise splenectomy at much earlier periods.

Laboratory Data. The increased rate of red cell destruction produces anemia, hyperbilirubinuria, an elevated icterus index, increased fecal and urinary urobilinogen, and elevated serum iron concentration. The increased rate of red cell regeneration leads to reticulocytosis. The blood picture is characterized by anemia, spherocytosis, increased osmotic fragility, and an elevated serum bilirubin. The Coombs test is negative, and an abnormal hemoglobin is absent. The hemoglobin level may be normal but usually ranges from 7 to 11 gm per 100 ml, and the red blood cell count ranges from 2.5 to 4.5 million per cubic millimeter. During a crisis the hemoglobin may fall to 3 to 4 gm, and the red cell count to 1.5 to 2 million. The reticulocytes range from 5 to 20 per cent.

The spherocytes appear as small, bright, deeply stained cells which lack central pallor. Their decreased diameter and increased thickness contribute to the rounded appearance. Normoblasts may appear in the peripheral blood. Poikilocytosis is rare. Large polychromatophilic cells are common and appear in the blood smear in conjunction with normal and spheroidal cells. Spherocytes may be few in number or constitute the bulk of cells. Except in the mildest cases the osmotic fragility is increased. When this is uncertain, fragility is accelerated by prior incubation of the blood for twenty-four hours at 37° C. Mechanical fragility is increased. The autohemolysis test reveals greater than normal lysis of the red cells during sterile incubation at 37° C. for forty-eight hours. The addition of glucose causes marked inhibition of autohemolysis.^{104,105} The leukocytes are usually normal in number or may be slightly increased. The platelets are normal. Bilirubinemia varies between 1 and 4 mg per 100 ml, but often lies within the normal range despite other signs of active hemolysis.¹⁶

A normal or slightly lowered hemoglobin concentration may coexist with active hemolytic disease. An abnormally small total red cell volume may be present in such cases and in combination with a rapid rate of red cell production results

in a partially compensated hemolytic anemia.⁴ Whether or not anemia exists depends upon the ability of red cell production to compensate for the increased destruction of cells. The failure of the plasma volume to expand accounts for the apparently normal hemoglobin level in the peripheral blood. As yet there is no known explanation for the lack of expansion of the plasma volume in patients with this disease or the greater than normal expansion in patients with sickle cell anemia.

Since hereditary spherocytosis is characterized by a chronic and persistent hemolytic process, one would expect episodes of exaggerated hemolysis to occur. In contrast to an anticipated increase in hemolysis, the crisis in hereditary spherocytosis is now known to be aplastic. Instead of a hyperplastic bone marrow which is ordinarily present in this disease, an acute aplasia of erythroid tissue supervenes and is associated with reticulocytopenia, anemia, leukopenia, and thrombocytopenia. The serum bilirubin decreases and jaundice gives way to pallor.

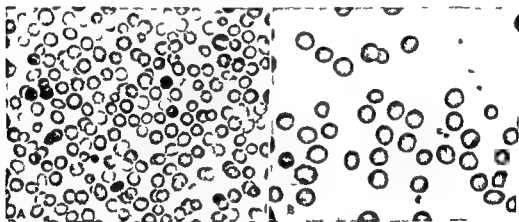


FIG. 10 Blood smear of a patient with hereditary spherocytosis. A Low power view showing dark staining of abnormally thick spherocytes ($\times 250$). B Slightly increased magnification of A.

Hereditary Spherocytosis in the Neonatal Period Hereditary spherocytosis may be manifest at birth.^{96, 106, 113} The blood smear in the neonatal period shows both macrocytic cells from fetal life and spherocytes. The latter become increasingly numerous in the weeks following birth. Excessive jaundice due to the accumulation of indirect and unconjugated bilirubin leads to confusion with erythroblastosis. The differentiation from cases of erythroblastosis due to A and B blood groups which are also characterized by spherocytosis may be difficult. The persistence of spherocytes and a negative Coombs test in patients with hereditary spherocytosis separate the two conditions. At any rate exchange transfusions are required in patients with hereditary spherocytosis in the event of hyperbilirubinuria since the same susceptibility to nervous system damage exists as in patients with erythroblastosis.

It is noteworthy that in patients with hereditary spherocytosis several supportive transfusions may be required during the first three weeks of life be

cause of severe anemia. Thenceforth patients attain a steady state in which the anemia is minimal and symptoms are few.⁴⁰

Diagnosis The important diagnostic features of hereditary spherocytosis stem from the intrinsic red cell anomaly. As has already been indicated, the morphology of the red cells accounts for the increased osmotic fragility, reticulocytosis, jaundice, and splenomegaly. Spherocytosis and increased osmotic fragility are not exclusive features of hereditary spherocytosis and may be present in patients with other hemolytic disorders. A positive family history and one in which relatives manifest these hematologic features contribute to the diagnosis. Incubation of blood samples at 37° C. for twenty-four hours increases osmotic fragility in the asymptomatic and affected relative or the patient with the mild or latent disease. Diagnosis may be difficult in the child without a positive family history or in one who is initially observed in an aplastic crisis.

It should be emphasized that the absence of jaundice and absence of anemia are inadequate criteria for the diagnosis of a latent case or a spherocytic trait. Jaundice and bilirubinemia are largely dependent upon the excretory capacity of the liver since hemoglobin and red cell levels depend not only upon the rate of hemolysis but also upon the capacity for regeneration. Thus determination of fecal urobilinogen excretion may provide evidence of a marked hemolytic disorder in the absence of jaundice or hyperbilirubinemia. The reticulocyte count may reveal accelerated red cell regeneration in the absence of anemia. This determination is especially important in patients in whom the failure of the plasma volume to expand in the face of a diminished red cell mass results in a normal or slightly lowered hemoglobin level in the peripheral blood.

Autoimmune acquired hemolytic anemia usually shows marked spherocytosis but the positive Coombs test and negative family history differentiate it from hereditary spherocytosis. In patients with hereditary nonspherocytic anemia spherocytes are absent and osmotic fragility is normal.

Treatment Splenectomy is followed by a complete and permanent relief from all signs and symptoms of the disease. Except for the asymptomatic patient in whom red cell destruction is well compensated, the operation is recommended once the diagnosis is established. Even in the mildly affected person without anemia or jaundice, splenectomy is advisable for the relief of a chronic hemolytic condition and proneness to crises and gallstone formation. Because of evidence that splenectomy in early childhood may cause increased susceptibility to infection,⁴¹ it has been suggested that the operation be postponed until the patient is at least 5 years of age when gallstones tend to be formed.^{1,4} Occasionally an earlier operation at approximately 2 years of age is necessary when anorexia and fatigability seriously interfere with the health of the child. By pooling data from reported sources from 1934 to 1957 of the evidence of infection following splenectomy for hereditary spherocytosis in the first year of life, Burman⁴² found that 10 to 21 per cent of the forty-eight patients developed an infection and that 4 to 8 per cent died. It would seem that splenectomy in the first year of life is definitely hazardous. The optimal age for the operation still awaits the results of more extended experience. Children require close supervision for several years postoper-

in a partially compensated hemolytic anemia.⁴ Whether or not anemia exists depends upon the ability of red cell production to compensate for the increased destruction of cells. The failure of the plasma volume to expand accounts for the apparently normal hemoglobin level in the peripheral blood. As yet there is no known explanation for the lack of expansion of the plasma volume in patients with this disease or the greater than normal expansion in patients with sickle cell anemia.

Since hereditary spherocytosis is characterized by a chronic and persistent hemolytic process, one would expect episodes of exaggerated hemolysis to occur. In contrast to an anticipated increase in hemolysis, the crisis in hereditary spherocytosis is now known to be aplastic. Instead of a hyperplastic bone marrow which is ordinarily present in this disease, an acute aplasia of erythroid tissue supervenes and is associated with reticulocytopenia, anemia, leukopenia, and thrombocytopenia. The serum bilirubin decreases and jaundice gives way to pallor.

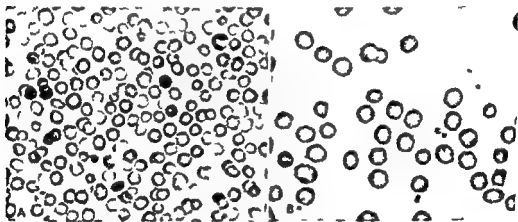


FIG. 10. Blood smear of a patient with hereditary spherocytosis. A. Low power view showing dark staining of a normally thick spherocyte ($\times 50$). B. Slightly increased magnification of A.

Hereditary Spherocytosis in the Neonatal Period Hereditary spherocytosis may be manifest at birth.^{1, 113} The blood smear in the neonatal period shows both macrocytic cells from fetal life and spherocytes. The latter become increasingly numerous in the weeks following birth. Excessive jaundice due to the accumulation of indirect and unconjugated bilirubin leads to confusion with erythroblastosis. The differentiation from cases of erythroblastosis due to A and B blood groups which are also characterized by spherocytosis may be difficult. The persistence of spherocytes and a negative Coombs test in patients with hereditary spherocytosis separate the two conditions. At any rate exchange transfusions are required in patients with hereditary spherocytosis in the event of hyperbilirubinuria since the same susceptibility to nervous system damage exists as in patients with erythroblastosis.

It is noteworthy that in patients with hereditary spherocytosis several supportive transfusions may be required during the first three weeks of life be

A familial incidence is well documented¹⁹⁻²¹ but, as in patients with hereditary spherocytosis it can not always be demonstrated in the asymptomatic siblings or parent. Sickle cell anemia, Cooley's anemia, and hereditary spherocytosis are readily differentiated by clinical and hematologic criteria.

Increasing evidence indicates that hereditary nonspherocytic hemolytic anemia is not a single entity and that not all patients with this disease suffer from the same disorder. The disorders are similar with respect to a shortened red cell survival, normal osmotic fragility, failure to respond to splenectomy, and often development of hyperbilirubinemia and anemia shortly after birth. At least two distinct types have been separated on the basis of the extent of increased osmotic fragility and autohemolysis (with or without added glucose) after twenty-four hours of incubation at 37°C.²² In patients with one of these types the greatly increased osmotic fragility and the failure of glucose to diminish autohemolysis, as occurs in patients with normal blood, suggest a metabolic defect.²³ In patients with another type the red blood cells were found to possess the properties of cells sensitive to primaquine—namely, a reduced glutathione content, a markedly abnormal glutathione stability test, and an extreme reduction of glucose-6-phosphate dehydrogenase. In patients with this type the morphology of the red cells is normal and the degree of anemia mild except during infections. The defect is present in other members of the family and in several generations.

Hereditary nonspherocytic hemolytic anemia should be considered in patients who present the clinical picture of erythroblastosis at birth without the characteristic laboratory findings of the latter disease.¹⁹ Hyperbilirubinemia of the indirect type in the first days of life necessitates exchange transfusion as in erythroblastosis before critical levels are reached. It is characteristic however of hereditary nonspherocytic anemia that chronic anemia persists following discharge of the patient from the hospital. The diagnosis is often made by exclusion from a heterogeneous variety of intrinsic blood disorders.

Frequent transfusions to maintain hemoglobin levels of approximately 7 gm per 100 ml constitute the only reliable form of therapy. Excessive blood requirements may lead to an extracorporeal hemolytic defect,²⁴ in which case splenectomy is beneficial although the basic hemolytic process remains unaltered. The congestion with spherocytes which is characteristic of the spleen of the patient with hereditary spherocytosis is strikingly lacking in the splenic pulp of the patient with nonspherocytic anemia although hemosiderin deposits are abundant.

Hereditary Elliptocytosis With Hemolytic Anemia

Hereditary elliptocytosis is an uncommon hereditary anomaly characterized by the presence of large numbers of oval and elliptical cells in the peripheral blood and is usually discovered on routine examination.²⁵ It is transmitted as a mendelian dominant and either sex may be equally affected. Elliptocytes are usually found in one parent but despite this unilateral inheritance about 12 per cent of the offspring bearing the trait²⁶ show evidences of a hemolytic anemia. In only a few reported cases have both parents been affected and in these the offspring gave evidence of a severe hemolytic anemia.²⁷⁻²⁹ At birth elliptical red cells are few in number and do not appear in significant numbers until the third to fourth month of life although jaundice and anemia may be present in the first

tively so that immediate and energetic treatment can be instituted in the event of sudden and severe illness.

Spherocytosis as well as increased osmotic fragility of the red corpuscles persist after splenectomy. A sharp rise in platelets and leukocytes follows postoperatively. The reticulocyte count drops to normal but is occasionally slightly elevated. Hemolysis ceases and the bilirubin level falls significantly. The life span of the red cells previously shortened becomes approximately normal after splenectomy.

Blood transfusion is required in the early months of life until the hemoglobin stabilizes and for treatment of a crisis with severe anemia.

Sporadic Congenital Spherocytosis Associated With Congenital Hypoplastic Thrombocytopenia and Malformations

A syndrome characterized by congenital malformations (such as absent radius, cyanotic heart disease, and bilateral hydronephrosis), thrombocytopenia, hypoplasia of megakaryocytes, and the sporadic form of spherocytic anemia has been described in infants.¹⁻³ The spleen may be transiently enlarged and petechiae may be present in early infancy. A follow-up into childhood is not yet available in these patients. Except for the presence of spherocytes, the combination of depleted megakaryocytes and platelets dating from the neonatal period resembles congenital hypoplastic thrombocytopenia. In many respects the clinical and hematologic features constitute a variant of Fanconi's syndrome to the extent that a severe anemia coexists with congenital abnormalities.

Hereditary Nonspherocytic Hemolytic Anemia (Atypical Familial Hemolytic Anemia, Congenital Nonspherocytic Anemia)

Hereditary nonspherocytic hemolytic anemia applies to a form of chronic hemolytic anemia frequently familial and transmitted as a mendelian dominant in which the red cells possess an intrinsic defect without a manifest morphologic anomaly. In this latter respect it differs from hereditary spherocytosis, sickle cell anemia, and thalassemia in which an intrinsic defect coexists with a distinctive cellular malformation. Osmotic and mechanical resistance of the red cells is normal and the life span of the red cells is shortened (twelve to seventeen days²¹). An abnormal type of hemoglobin has not been demonstrated. In contrast to hereditary spherocytosis there is not only normal red cell fragility but also a failure to respond to splenectomy. The anemia varies in intensity from mild to severe requiring transfusions. The red cells are normocytic but show a tendency toward microcytosis and occasionally ovalocytes are present.¹ The hematologic picture includes erythroid hyperplasia of the bone marrow, reticulocytosis, normoblasts in the peripheral blood, hyperbilirubinemia, and punctate basophilia of the erythrocytes.²⁻⁴ Fecal urobilinogen is increased. The Coombs test is negative. Autohemolysis and osmotic fragility after incubation at 37° C. for twenty-four hours showed a slight increase over the normal. Clinical features include mild jaundice, slight to moderate enlargement of the liver and spleen and in rare instances facial configuration of the mongoloid type. Thickening of the cranial bones and a hair on end appearance between the tibiae similar to that observed in patients with Cooley's anemia and sickle cell anemia have been reported.^{1,22}

Diagnosis is made by the presence of elliptical cells in the patient in a parent and in other members of the family. Thalassemia minor in which elliptical cells are also commonly seen may be confused with elliptocytosis.³ Elliptocytosis has been reported in association with other types of disc disease among them the sickle trait.⁴

Treatment Patients who are asymptomatic or give evidence of compensated hemolytic disease require no treatment. In addition to supportive treatment by transfusions splenectomy has benefited most patients with severe uncompensated anemia.^{5,6} Although red cell destruction is reduced the characteristic oval and rod shaped elliptical cells persist.

Hemolytic Anemia Due to Enzyme Deficiency Following Administration of Drugs and Other Agents

The recent studies on primaquine sensitivity have brought to light intrinsic defects in red cells rendering them susceptible to hemolysis. These abnormalities are apparently harmless unless red cells are exposed to primaquine and other drugs commonly used in medicine.⁴ It was shown that in malarial primaquine induced intravascular hemolysis in about 10 per cent of American Negroes but rarely in Caucasians. After approximately 50 per cent of the original cells are hemolyzed the younger population of red cells is resistant to further hemolysis and the anemia is thus self limited.⁴¹

Increasing evidence emphasizes the importance of glutathione in maintaining red cell integrity and in allowing activation of enzyme systems through its reducing or protecting action.⁴² The characteristics of the primaquine sensitive erythrocytes are a decreased content of reduced glutathione, marked fall of glutathione after incubation of whole blood with acetylphenylhydrazine (glutathione stability test) and a deficiency of glucose 6-phosphate dehydrogenase. Both in vivo and in vitro exposure of the red cell to a drug of the primaquine type causes a further fall in the already decreased glutathione content.⁴

Glutathione exists in a reduced form (GSH) and an oxidized form (GS-SG). The interconversion of these two is catalyzed by an enzyme glutathione reductase which requires reduced triphosphopyridine nucleotide (TPNH) for the reduction of glutathione. One of the principal sources of the required TPNH (which serves as a coenzyme) is the oxidation of glucose 6-phosphate to 6-phosphogluconic acid (successive intermediates in the direct oxidation of glucose) which is catalyzed by an enzyme glucose 6-phosphate dehydrogenase. It is this enzyme which is deficient in the red cells of persons sensitive to primaquine.⁴ When this source of TPNH is unavailable because of enzymatic deficiency the red cell is unable to maintain its glutathione in the reduced state.

The glucose 6-phosphate dehydrogenase is in highest concentration in the younger cells of both sensitive and nonsensitive population and is actually higher in the very young cells of the sensitive population than in the oldest cells of the nonsensitive population. The young cells of the sensitive population therefore are resistant to hemolysis since they have enough enzyme to generate sufficient TPNH to keep the glutathione reduced. The defect in glutathione metabolism expresses itself in the older erythrocytes.

The red blood cells of susceptible persons who have a low glutathione level show an increased tendency to form Heinz bodies. The Heinz bodies are visible

month.¹¹¹³ The nucleated precursors of the elliptical cells in the marrow are round with the abnormal cells making their initial appearance in the reticulocyte stage or later. No abnormal hemoglobin has been detected in the cells of patients with hereditary elliptocytosis. The gene determining elliptocytosis is located in the same chromosome as that carrying the genes for the Rh blood groups.¹¹¹⁴ There is no sickling in wet preparations.

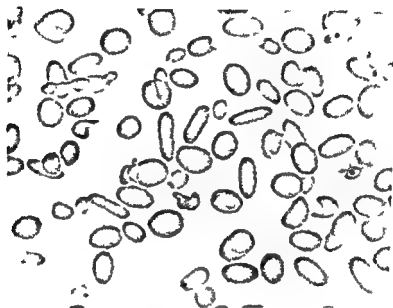


Fig. 11 Photomicrograph of blood smear of patient with hereditary ovalocytosis ($\times 1200$). Note larger number of elliptical, oval, and elongated cells.

Clinical and Blood Findings The characteristic cells are oval, elliptical, sausage shaped, elongated, and rod shaped,⁴ and together constitute over 50 per cent of the red cell population. Osmotic resistance is normal except in patients with overt hemolytic anemia in whom it is decreased. The condition is usually benign without symptoms or signs of anemia notwithstanding the presence of elliptical cells in the blood. In the patient with the severe hemolytic form the peripheral blood shows ovalocytes, elliptocytes, microspherocytes, distorted microcytes, and fragmented forms. Small numbers of oval cells (1 to 15 per cent) may appear in the blood of normal persons.⁴ They are often found in increased numbers symptomatic of disorders other than hereditary elliptocytosis. Such are conditions in which anisocytosis and poikilocytosis are prominent as in patients with iron deficiency anemia, mild and severe Cooley's anemia, macrocytic anemia, and severe anemias associated with leukemia and cancer.⁹

In the uncommon hemolytic cases two grades of severity are observed: one with hemolysis without anemia and the other with hemolytic disease and anemia.⁹ In both types splenomegaly, hematologic evidences of hemolysis, and bone marrow regeneration make their appearance, and the life span of the red cells, usually normal, is shortened.¹¹¹⁵

attacks of hemoglobinuria are marked by the passage of dark urine occurring during sleep regardless of the time of day. The disease occurs most commonly in adult life (usually the third decade) but a few cases have been described in children.^{81,82} It is due to an inherent red blood cell defect presumably an abnormal stromal protein which makes the cells susceptible to hemolytic factors present in both the serum and plasma of normal persons and of the patient. These factors include complement, thrombin,¹ and properdin.³ Properdin is a natural heat labile serum protein which in association with magnesium and complement is involved in the destruction of bacteria and viruses.² The exact mode of action of these factors in hemolysis is complex and not yet established. The coagulation system also appears to be involved in some way in the precipitation of a hemolytic crisis. The red cells show a shortened survival when injected into normal subjects. Markedly reduced erythrocyte acetylcholinesterase activity which is localized to the stroma has been demonstrated in patients with this disease. This impairment possibly diminishes the integrity of the red cell.¹⁶

The patients are persistently anemic and slightly icteric and show a slight to moderate enlargement of the liver and spleen. They are fatigued, complain of headaches and abdominal and lumbar pain and are febrile during a hemolytic crisis. Hemoglobinuria, hemoglobinemia and hemosiderinuria which characterize the disease are evidence of intravascular hemolysis.

The blood picture reveals an anemia of varying severity with moderate reticulocytosis (10 to 20 per cent), polychromasia and macrocytosis. There is a tendency toward neutropenic leukopenia and thrombocytopenia. Spherocytes are not present and osmotic fragility is not increased. The bone marrow is hypercellular indicative of increased regeneration and rarely are regenerative. The serum bilirubin does not exceed 3 mg per 100 ml. A tendency toward thrombosis in the systemic (including cerebral) and portal circulations accounts for varied symptomatology. The increased rate of hemolysis produced by lowering the pH of the blood has suggested that the mechanism of the disease is related to an accumulation of acid metabolites during sleep inducing hemoglobinuria.

In the differential diagnosis the lack of a history of cold precipitating hemolysis eliminates paroxysmal cold hemoglobinuria. Acute acquired hemolytic anemia (Lederer's anemia) in which hemoglobinuria is also present occurs commonly in children and is unaccompanied by pancytopenia or a congenital defect of the red cells.

The course is chronic of varying severity with occasional exacerbations often precipitated by infections. Death from anemia, thrombosis and infection may occur. Prolonged survival as well as spontaneous cures has been reported.

The diagnosis is confirmed by the Ham acid serum tests.⁸ In a positive test the patient's cells undergo hemolysis in acidified normal serum at 37° C. Additional lysis of patient's cells can be achieved by addition of thrombin to the test.

There is no specific treatment. Splenectomy is disappointing and potentially hazardous. Transfusion is the most beneficial form of treatment. Because of the possible hemolytic effect of plasma factors on the patient's erythrocytes, washed red cell suspensions have been employed with good results.

manifestations of oxidative damage to either hemoglobin and/or the stroma of the sensitive cells.¹ The chemical relationships between the drug and the oxidation of glutathione and between the oxidation state of the glutathione and hemolysis are not clear.

Similar mechanisms account for hemolytic anemia from exposure to fava beans,²¹⁰ naphthalene,^{1, 4} and vitamin K analogues.^{1, 14} Cells sensitive to primaquine have also been shown to be unusually susceptible to hemolysis by certain aniline derivatives including sulfanilamide, phenacetin, Promizole, and acetanilid.⁴⁰ The fact that erythrocytes of sensitive persons are more susceptible to the *in vitro* Heinz body formation reaction than are cells of nonsensitive persons serves as a valuable means of predicting primaquine sensitivity.

A high proportion of symptomatic parents and siblings of the affected person with a history of favism or hemolytic anemia due to drugs have a low glutathione content in their red cells,¹¹⁰ and a deficiency of glucose 6-phosphate dehydrogenase.⁶¹ In a survey of randomly selected Negroes the incidence of persons sensitive to primaquine based on the glutathione stability test was found to be about 14 per cent among males and 2 per cent among females.¹ Among relatives of "revertors" males also predominated. The defects are apparently transmitted by a sex-linked gene with incomplete dominance.

The defect in glutathione metabolism has been thoroughly investigated in relation to naphthalene-induced hemolytic anemia in infants and children.^{20, 14} Only a small percentage of children who have ingested naphthalene moth balls develop a hemolytic anemia and these are most often Negroes. Abnormal numbers of Heinz bodies also appear in the cells after incubation. The hemolytic effect is attributed not to naphthalene itself but to its derivatives. Transplacental passage of these compounds was noted in one case¹⁴ in which both the mother and the newborn infant showed evidence of a hemolytic anemia after the mother ingested moth balls.

A similar mechanism (glutathione instability) has been postulated for hemolysis occasionally encountered in newborn infants who are given excessive amounts of vitamin K at birth.¹

From the group of diseases classified as hereditary nonspherocytic hemolytic anemia one variety has also been found to possess the properties of primaquine-sensitive erythrocytes. These cells show a reduced glutathione content, markedly abnormal glutathione stability, and in extreme reduction of glucose 6-phosphate dehydrogenase. In this group similar defects also appeared in the families of these patients.^{10, 11} That a familial defect is not always in evidence was noted in a patient with congenital nonspherocytic hemolytic anemia in whom the disease manifested itself in the absence of G6PD but was accentuated by the ingestion of this drug.²⁴

Nonhereditary Hemolytic Anemia—Paroxysmal Nocturnal Hemoglobinuria (Marchiafava-Micheli Syndrome)

Paroxysmal nocturnal hemoglobinuria is an uncommon type of chronic hemolytic anemia with an insidious onset and is characterized by attacks of hemoglobinuria occurring mainly at night. It is neither congenital nor hereditary. The

antibodies. In patients with the hemolytic anemia caused by the agents enumerated previously they are only irregularly found. In persons with autoimmune hemolytic anemia, however, these antibodies play a dominant role in pathogenesis and constitute one of its major features.

Autoimmune Hemolytic Anemia (Chronic Idiopathic Autoimmune Hemolytic Disease, Chronic Acquired Hemolytic Anemia)

Autoimmune hemolytic anemia is characterized by the presence of demonstrable abnormal antibodies in the blood which are produced by the body and are directed against its own red cells. The causation may be unknown and hence be idiopathic or symptomatic of an underlying disorder. The latter disorders include chronic leukemia (mainly chronic lymphocytic leukemia), the lymphomas (Hodgkin's disease, follicular lymphoblastoma, reticulum cell sarcoma and lymphosarcoma), disseminated lupus erythematosus and other collagen diseases (scleroderma, polyarteritis nodosa, dermatomyositis), neoplasms and liver disease. Autoimmune hemolytic anemia also occurs with virus pneumonia in the second or third week of illness and after the onset of infectious mononucleosis.

Clinical Features. Autoimmune hemolytic anemia occurs at all ages and has been reported in an infant 5 months old.² The disease is usually chronic with an insidious onset and is prone to exacerbations of severe hemolytic anemia. In children pallor, lethargy, fatigue, anorexia and low grade fever are common. Mild to moderate jaundice is present in about 75 per cent of the patients. The spleen is usually palpable and varies in size when it is greatly enlarged an underlying disease should be suspected. Moderate enlargement of the liver is frequent and lymphadenopathy is absent. Purpura is indicative of a secondary condition. Hemoglobinuria is unusual and is an occasional accompaniment of a hemolytic episode.

A type of autoimmune hemolytic anemia has been described in which a depression of red cells and reticulocytes in the peripheral blood is associated with a temporary disappearance of erythroblasts from the bone marrow.⁴⁴⁻⁵ This is explained by antibodies acting peripherally on erythrocytes and reticulocytes and in the bone marrow on the erythroblasts.

Blood Findings. The blood picture varies with the extent of the anemia and the degree of bone marrow compensation. The hemoglobin usually ranges between 10 and 11 gm. per 100 ml. but may drop to levels of 3 to 5 gm. per 100 ml. in an acute attack. The blood smear shows a persistent reticulocytosis (up to 60 per cent in severe hemolytic episodes), polychromatophilia, microspherocytosis, anisocytosis and often a tendency toward macrocytosis. Spherocytosis is marked in the active phase and less noticeable when the disease is quiescent. Normoblasts appear in small numbers. Erythrophagocytosis by monocytes and sometimes by neutrophils may be seen in the blood smear and in greater numbers from the buffy coat of incubated venous blood.¹⁻³ The polymuclear leukocyte count varies during chronic periods and may be greatly elevated during an exacerbation. In one of our patients the total leukocyte count rose to 11,300 per cubic millimeter. The platelet count is usually normal but may be depressed. Thrombopenia has been inter-

ACQUIRED HEMOLYTIC ANEMIAS

The acquired hemolytic anemias embrace a group of heterogeneous disorders of varying etiology whose pathogenesis is dependent on an extracorporeal mechanism rather than on an intrinsic defect of the red cell. They are classified as immune or nonimmune depending upon whether or not an immune mechanism can be demonstrated. The immune group comprises those conditions in which antibodies contribute to the pathogenesis. They include autoimmune hemolytic anemia, erythroblastosis fetalis, transfusion reactions, and paroxysmal cold hemoglobinuria.

The nonimmune acquired hemolytic anemias appear after treatment with drugs such as quinine, phenylhydrazine, and acetylphenylhydrazine, sulfonamides, and arsenical compounds; after poisoning caused by chemicals such as naphthalene, benzene, and nitrobenzene; and after exposure to physical agents such as extreme heat or cold. They may follow infection by bacteria, bacterial products, viruses, and parasites. The action of these agents is characterized by direct destruction of erythrocytes or inhibition of immature forms in the bone marrow. Obviously, treatment necessitates elimination of the offending drug, chemical, or toxic agent.

Recent investigations have shown (p. 228) that such agents as sulfonamides, naphthalene, primaquine, and vegetable poisons (for example fava bean) owe their action to a familial and intrinsic defect in the red cell which renders it susceptible to destruction. It is to be expected that other anemias in this group will eventually disclose the same relationship. In the case of favism, ingestion of even small amounts of fava bean or inhalation of the pollen from the blossom of the plant produces in sensitive persons, especially children, a sudden acute attack of hemolysis accompanied by jaundice and often hemoglobinuria. The episode usually lasts less than a week and responds to transfusions. Hemoglobinuria occurs in severely burned patients with evidences of fragmented red cells, spherocytes, and increased susceptibility to osmotic and mechanical fragility.⁶³ The red cells with a shortened life span appear to be destroyed intravascularly.

Malarial infection produces anemia by toxic inhibition of the marrow and actual parasitization of the red cells by the protozoa. The fulminating hemoglobinuria and severe associated clinical symptoms which occur in the condition known as blackwater fever occur in the majority of patients in the course of infection by *Plasmodium falciparum*. Infection by organisms such as *Clostridium welchii* and *Bartonella bacilliformis* (in one of its chimeric forms) is characterized by severe hemolytic anemia (Oroya fever). In this infection, which occurs in inhabitants of the central zones of Peru, Colombia, and Ecuador, numerous organisms invade the blood stream, and stained smears reveal as many as 90 per cent of the erythrocytes to be heavily invaded.

The immune acquired hemolytic anemias reveal antibodies in the blood which are detected by the Coombs (antiglobulin) test. These antibodies are globulins which are elaborated by the patient through some unknown mechanism and lead to premature destruction of the red cells—hence the designation of autoimmune

hemolytic anemia with the loss of normally acquired tolerance and the development of antibodies to the patient's own red cells.¹¹³

The simultaneous attacks on the different blood elements in the same destructive process was emphasized by Evans and co-workers^{45, 49} who observed that acquired hemolytic anemia with sensitization of the red cells is often accompanied by thrombocytopenia. Primary thrombocytopenia in turn frequently coexists with red cell sensitization with or without hemolytic anemia.

The formation of autoantibodies has also been ascribed to other mechanisms³ an alteration of patient's erythrocytes making them foreign to his own antibody forming mechanism and thus permitting the development of anti red cell antibodies or the formation of abnormal proteins by the patient with an abnormal reaction on the part of his antibody forming tissue. False positive Wassermann and Kahn tests sometimes found in patients with this disease fit in with the latter hypothesis.³¹

Destruction of Sensitized Red Cells The spleen, liver⁶ and other portions of the reticuloendothelial system contribute by as yet unknown mechanisms to the destruction of red cells coated by antibodies. Much evidence has isolated the spleen as the important organ in which sensitized red cells are filtered from the circulation, trapped and sequestered, phagocytosed and lysed.^{31, 1} Spherical red cells are particularly susceptible to lysis.¹¹⁴

Diagnosis The diagnostic features of autoimmune acquired hemolytic anemia consist of anemia, reticulocytosis, jaundice, splenomegaly and elevated serum bilirubin with a positive Coombs test. A positive Coombs test in patients with acquired hemolytic anemia in contrast to the negative test in those with hereditary spherocytosis is helpful in differentiating the two conditions since spherocytosis and reticulocytosis occur in both. In all patients with hemolytic anemia with a positive Coombs test search should be made for one of the underlying diseases previously enumerated.

Treatment Steroid hormones, blood transfusions and splenectomy constitute the basis of treatment.

Adrenocortical Steroids Cases of autoimmune acquired hemolytic anemia and often symptomatic cases usually respond favorably to hormonal therapy. Permanency of improvement with these agents has not yet been thoroughly ascertained. The steroid hormones either alone or combined with splenectomy represent the treatment of choice. As with other blood disorders in which steroid hormones are used, dosage must be individualized.

Prednisone or prednisone like compounds are most commonly given initially in a total daily dosage varying from 40 to 60 mg. (for precise dosage and variety of corticosteroids see Chapter 23). When the response of the patient and the severity of the disease are ascertained the dosage may be adjusted accordingly. In favorable cases the dose is reduced within a week. This is marked by a fall in the reticulocyte count, a rise in the number of red blood cells and hemoglobin and a reduction in the number of transfusions and their elimination. Once a remission is obtained the steroid hormone is gradually reduced to a maintenance dose which in our experience is approximately 15 mg. daily. With relapse the initial dose is restored. In spite of improvement a positive Coombs test may persist

pected is part of the immune antibody mechanism in which the red cells are intensely involved.^{18,19}

Osmotic fragility and autohemolysis are both increased and parallel the degree of spherocytosis. An aplastic crisis indicated by reticulocytopenia, leukopenia and erythrocytic aplasia of the bone marrow is rare. The major fraction of increased hemolysis occurs in normal extravascular sites with the formation of excessive amounts of bile. With unimpaired liver function the bilirubin level is not greatly elevated and usually ranges from 1 to 4 mg. in quiescent periods.

Serologic Findings. Three different autoantibodies affect the red cells in autoimmune hemolytic anemia: autoagglutinins, autohemolysins, and incomplete antibodies permitting agglutination by antoglobulin serum (Coombs test). The blood of patients with autoimmune hemolytic anemia commonly undergoes rapid spontaneous autoagglutination after withdrawal from the body, resulting in clumping of the red cells so that blood counts are difficult. The autoantibodies may be nonspecific and therefore act as panagglutinins and panhemolysins. They react with normal erythrocytes in addition to the patient's own cells regardless of blood group or type. In some cases the serums react specifically and usually with well recognized blood group antigens within the Rh system.²¹

The antibodies may be absorbed on the surface of the red cells in which case they are detected by the direct Coombs antoglobulin test or are free in the serum. Their presence in serum may be demonstrated by the indirect Coombs technique in which normal red cells are initially exposed to the patient's serum before testing with the Coombs antoglobulin reagent. In neither case does the intensity of the Coombs test bear any relationship to the severity of the disease.

Two types of autoantibodies can be distinguished by their behavior in vitro: a warm type active at 37° C. (the more common in the idiopathic autoimmune disease)²¹ and the clinically infrequent cold type active at temperatures of 4° C. High titer cold agglutinins are potentially hemolytic.²⁰ Both types give positive direct antoglobulin (Coombs) reactions. Autoagglutination by cold antibodies is abolished at 37° C. Anemia, reticulocytosis and spherocytosis are not as conspicuous as in the patient with the hemolytic anemia with warm agglutinins. Hemolytic anemia associated with high titer cold hemagglutinins are principally found in patients with virus pneumonia. In older persons they are also active in the development of a rare form of chronic hemolytic anemia with hemoglobinuria, high titer cold hemagglutinins, and howards phenomenon.^{16,114} Cold antibodies are also involved in the pathogenesis of pyroximal cold hemoglobinuria. The Donath Landsteiner test which is diagnostic of this disorder requires cold for the union of antibodies with the red blood cells and warmth together with complement for eventual lysis. The titer of cold agglutinins is low in comparison with the elevated titer of agglutinins in patients with viral pneumonia.

Pathogenesis. It is of interest that the body in health avoids forming antibodies against its own tissues, a tolerance developed from early fetal life.¹⁵ Such dire circumstances in which a living organism becomes capable of producing antibodies against its own body components has been termed "horror autotoxicus" by Ehrlich.¹¹⁵ This situation apparently exists in autoimmune

toward spontaneous recovery and relatively recent introduction of hormone therapy. Fatalities have occurred in a relatively small number of children.⁹

Paroxysmal Cold Hemoglobinuria

In paroxysmal cold hemoglobinuria, a rare syndrome, sudden attacks of intravascular hemolysis and hemoglobinuria are precipitated by exposure to cold. The disorder is due to the presence of an autohemolysin in the patient's blood which is biphasic, uniting with the red cells at a low temperature and destroying them in the presence of complement upon subsequent warming up to body temperature. The Donath-Landsteiner test demonstrates this sequence of events by bringing together cells and serum in the cold and warming the suspension to allow complement to produce hemolysis. The direct antiglobulin (Coombs) test is positive at the height of a hemolytic episode and negative when the patient is symptom free.⁹ The Donath-Landsteiner antibody causes agglutination as well as hemolysis.⁹

The clinical and hematologic picture is that which characterizes acute hemolytic anemia, especially when hemolysis is intravascular: fever, a shaking chill, headache, pain in the abdomen and back, and the passage of dark, bloody urine. In addition to severe anemia and other features of hemolysis during the paroxysm, leukopenia and erythrophagocytosis appear,⁶² the latter serving to remove damaged erythrocytes. Paroxysmal cold hemoglobinuria occurs usually in patients with syphilis, especially congenital syphilis. Occasionally there is no clinical evidence of syphilitic infection, and a positive Wassermann or Kahn test is interpreted as being false. Treatment is directed toward the syphilitic infection when required and toward the avoidance of chilling. Transfusion of fresh whole blood is potentially harmful because this might result in adding complement to the patient's blood and thus provoke hemolysis of his own and the transfused cells.¹¹⁷

Dysproteinemias

The dysproteinemias represent a group of disorders characterized by an abnormality of the plasma proteins. They include cryoglobulinemias, hyperglobulinemias, and macroglobulinemias (Waldenström's syndrome). They have in common hemorrhagic manifestations. Although these conditions do not occur in the pediatric age group, one of these, cryoglobulinemia, may be mentioned here because of its association with hemolytic anemia.

Cryoglobulinemia. Cryoglobulinemia refers to the presence in the serum of an abnormal globulin (cryoglobulin) which precipitates spontaneously in the cold and redissolves on subsequent warming.^{80, 91} Cryoglobulinemia is usually secondary to another disorder such as multiple myeloma, leukemia, or malignant lymphomas. It is associated with purpura and Raynaud's phenomenon occurring after exposure to cold. Acute hemolytic anemia and high titer cold agglutinins have also been described.¹¹⁷

Acute Acquired Hemolytic Anemia (Lederer's Anemia)

Sporadic cases of acute hemolytic anemia, often referred to as Lederer's anemia,⁷⁸ occur most commonly in children. The condition is characterized

and should prompt in alertness to recurrences of the hemolytic state. If prednisone or a substitute fails to relieve a remission ACTH should be tried before or after splenectomy. In summary, steroid hormones in adequate dosage represent a remarkably effective form of therapy, especially in the initial control of this disorder.¹⁰

Transfusions Blood transfusions are given when the hemoglobin level drops below 7 to 8 gm per 100 ml and are repeated in accordance with the demands of the patient to maintain normal activity without discomfort. Transfusions represent supportive therapy but do not alter the hemolytic process. Although of temporary value they play an important role in tiding the patient over a severe hemolytic episode. In the severe case the transfused cells are rapidly destroyed and the hemoglobin rise is not maintained. The extrinsic mechanism in this disease (the autoimmune antibodies) destroys not only the patient's cells but also transfused normal cells as well.

The presence of autoantibodies frequently interferes with the typing and cross matching of blood. In these cases the least incompatible blood is utilized pending the effect of steroid hormones. Careful cross matching, slow administration of blood, and alertness to reactions are essential. Occasionally it is not feasible to maintain a hemoglobin at optimal levels and the patient is required to make appropriate adjustments until other modes of therapy are introduced. Replacement transfusion was successfully employed in a patient with severe hemolysis with dramatic although temporary response. The purpose of this procedure was to remove large amounts of antibody and antibody coated red cells.⁹

Splenectomy Prior to the advent of ACTH and corticosteroid therapy, great reliance was placed on splenectomy for treatment. Results were unpredictable, however, and the recovery rate when splenectomy was done as an initial procedure was estimated as approximately 50 per cent in patients with autoimmune hemolytic anemia. Permanency in the favorable case was extremely uncertain. Since autoantibodies are produced in large measure by the spleen, removal of this organ would be expected to result in a sharp decrease in their concentration. The variable effect of splenectomy, however, indicates that the spleen may not be the sole source of antibody.

At present splenectomy is reserved for patients in whom steroid therapy alone is inadequate, especially when excessively large doses of prednisone are required to control the hemolysis without inducing serious metabolic side effects. Following splenectomy, the resumption of steroid hormones is usually necessary. At present steroid therapy represents the treatment of choice and splenectomy should be reserved for those patients who fail to respond to hormone therapy.¹⁴

Prognosis The outlook in autoimmune acquired hemolytic anemia has greatly improved with the advent of steroid therapy. A complete and sustained remission rate of approximately 80 per cent has been obtained with the use of corticosteroids or corticosteroids plus splenectomy.¹⁰ Severe anemia, reticulocytopenia, thrombocytopenia and leukopenia are associated with a high mortality rate.⁸ The varied forms and combinations of therapy, however, still await a longer experience for ultimate evaluation. The effects of therapy are difficult to measure because of the uncertain course, remissions, exacerbations, tendency

Attacks recur in the same person after brisk exercise. The mechanism of hemoglobinuria is still unknown. Chilling of an extremity fails to produce the condition. A closely allied syndrome termed "exercise myohemoglobinuria" has been described in which painful swollen muscle groups and the excretion of dark urine after excessive exercise were the essential complaints.¹¹

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by its acute onset, short duration, and spontaneous recovery. Hemoglobinuria, jaundice, vomiting, prostration, restlessness, elevation of temperature, leukocytosis, and marked anemia are predominant features. Splenomegaly is inconsistent. Azotemia, oliguria, and anuria may be present, but death from renal failure is rare. The infrequency with which antibodies and a positive antiglobulin (Coombs) test are found is probably explained by the rapid course. The peripheral blood picture is one of a regenerative anemia with polychromatophilia, reticulocytosis, spherocytes, normoblasts, and erythrophagocytosis. Marked polynuclear leukocytosis (66,000 per cubic millimeter in one of our patients) with numerous metamyelocytes and myelocytes is indicative of increased bone marrow activity. In its subacute and milder form the disease may be difficult to differentiate from the autoimmune hemolytic disease with respect to the blood picture and serologic findings.²⁷⁻³¹ The acute hemolytic episode may continue in a milder form which is undistinguishable from the autoimmune disease. When tested at appropriate times antibodies can be demonstrated in as many as 50 per cent of patients.³¹

Despite the usual self-limited course, transfusions are required to maintain adequate hemoglobin levels. In the early stages when differentiation from an exacerbation of the chronic autoimmune disease has not yet been established, ACTH and adrenocortical steroids are usually given. Regardless of the precise diagnosis, these agents are of great value at the onset of the acute fulminating disease when the anemia is severe and the patient is in a state of severe collapse.

Idiopathic Paroxysmal Myoglobinuria

Hemoglobinuria must be distinguished from myoglobinuria. Idiopathic paroxysmal myoglobinuria is a rare disease of unknown etiology in which myoglobin is liberated from muscle and appears in the urine. Myoglobinuria can result from crushing injuries of muscle or can be idiopathic and unassociated with any specific or discernible cause. Severe muscle pain, especially in the calves, thighs, and back, spasm, weakness, or complete paralysis may result with the excretion of burgundy-colored urine.³²⁻³⁴ Myoglobin, having one-fourth of the molecular weight of hemoglobin, passes readily through the glomerular membrane. The disease is prone to recur and to be precipitated by exercise. Myoglobin and hemoglobin give a positive benzidine reaction. The absence of erythrocytes in the urine, the differences in the absorption bands observed by spectroscopic examination of the urinary pigment, and a negative test for porphyrin establish the diagnosis of myoglobinuria. The mortality rate is 30 per cent³⁴ with death caused by acute renal failure or respiratory paralysis.

March Hemoglobinuria

March hemoglobinuria is a rare and benign abnormality occurring in otherwise healthy young men in whom strenuous physical exertion carried out in the erect position is followed by the passage of dark urine.³ Hemoglobinuria is secondary to hemoglobinemia; jaundice, anemia, and reticulocytosis are absent. The condition has been observed in soldiers after marching, in marathon runners, and in college athletes. The patient recovers spontaneously within a few months.

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The Hereditary Hemoglobinopathies

The far reaching observations of Pauling and associates¹³ which demonstrated that the sickling phenomenon was dependent upon an abnormal hemoglobin stimulated an intense interest in human hemoglobin in normal and disease states. Rapidly accumulating information resulted in the identification of a large number of abnormal hemoglobins and showed that they were genetically controlled. This added information documented the concept that the clinical and pathologic features of many of the hereditary hemolytic disorders are basically conditioned by chemical and physical changes in the hemoglobin¹⁴⁻¹⁶.

The hemoglobinopathies comprise genetically determined disorders in which synthesis of normal adult hemoglobin is partially or completely suppressed causing the normal hemoglobin to be replaced by a hemoglobin variant including the fetal variety. *Thalassemia* is conveniently grouped with the hemoglobinopathies because of the large amounts of fetal hemoglobin replacing the normal adult type in patients with severe disease and the combination of fetal hemoglobin with other well-defined abnormal hemoglobins. No abnormal hemoglobin however has been described in patients with *thalassemia*.

The description of specific hereditary hemoglobinopathies necessitates a preliminary consideration of methods by which the abnormal hemoglobins are identified and an analysis of their genetic transmission.

Methods for Determining the Hemoglobin Types Differences between the hemoglobin of the human fetus and that of the adult have been known since 1886 when Korber¹⁷ demonstrated that fetal hemoglobin was more resistant to denaturation by alkaline solutions than was the adult type. Two simple procedures have served to identify the fetal and other types of hemoglobins. Fetal hemoglobin is determined by the method of alkali denaturation and the abnormal hemoglobins have been separated by the technique of paper electrophoresis.

Alkali Denaturation Method for Fetal Hemoglobin (Hemoglobin F) The fetal hemoglobin content is determined by the "one point" denaturation technique described by Singer and co-workers¹⁸. With this technique an approximate 10 per cent hemoglobin solution is denatured for exactly one minute by $\sqrt{12}\text{NaOH}$ the reaction being halted and the denatured hemoglobin precipitated by the addition of a 50 per cent saturated ammonium sulfate reagent. The hemoglobin

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a solution of veronal buffer (pH 8.6 some strength of approximately 0.06 and a specified voltage and current flow) the various hemoglobins migrate toward the anode according to their individual mobilities. After an initial period of two to three hours the hemoglobins have separated sufficiently to be recognized the length of the complete run (usually four to six hours) depending upon the particular requirements of the laboratory. At the end of the separation the strips are removed and dried and amounts of each hemoglobin may be roughly estimated or accurately measured.

Designation of Hemoglobin Types Recommended nomenclature designates the capital letter A for normal adult hemoglobin F for fetal hemoglobin and S for sickle cell hemoglobin and consecutive letters of the alphabet for abnormal types in order of their identification. Case reports have established the importance of types C, D, and E which occur in association with well defined hematologic entities. The racial incidence of hemoglobins is exemplified by the predominance of the abnormal hemoglobins S, C, and D in Negroes and E in natives of Thailand.

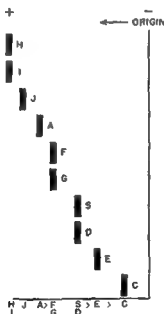


FIG. 12 Schematic representation of relative mobility of individual hemoglobins at pH 8.6 (From Smith C. H. The Abnormal Hemoglobins. Clinical and Hematologic Aspects. J. Pediat. 50:91, 1957.)

Electrophoretic Mobility of the Individual Hemoglobins According to the specific conditions prescribed for paper electrophoresis of the common hemoglobin types hemoglobins H and I move farthest toward the anode and C is the slowest remaining near the starting point. Intermediate between A and C in descending order of slower mobility are the hemoglobins F, G, S, D, and E.

concentration in the filtrate (undenatured) is determined photoelectrically and the percentage of undenatured or alkali resistant hemoglobin (fetal hemoglobin) is calculated

Jonxis and Huisman using another alkali denaturation technique¹⁰ in which readings are obtained from a curve constructed from sequential readings over a period of twelve minutes show a variance with the one minute denaturation method of Singer and co-workers. The former showed that at high percentages of fetal hemoglobin the results obtained with the one minute method are about 10 per cent too low. This is attributed to the fact that in completely denatured hemoglobin is removed by precipitation and filtration. At concentrations below 10 per cent of fetal hemoglobin this method is less accurate and more difficult to interpret. A combined alkaline denaturation and spectrographic method has also been described suitable for the range of 1 to 10 per cent fetal hemoglobin and indicates its presence at the trace level (0.4 to 1 per cent).

Electrophoresis The migration of charged particles in an electrolyte solution which takes place when an electric current is passed through the solution is termed electrophoresis. In this process particles bearing positive charges migrate to the cathode while negatively charged particles move to the anode. This principle has provided a means by which plasma proteins and more recently hemoglobin solutions exposed to an electric field under selected conditions are separated in accordance with their isoelectric points and the individual mobilities of each constituent. The speed and direction of forward migration depend upon the type of solution used to conduct the electric current its pH, the ionic strength and the strength of the electric current.

The moving boundary method of Tiselius previously extensively employed has been recently simplified with the use of paper electrophoresis. With the latter a strip of filter paper properly prepared permits the differentiation of serum proteins and of hemoglobins according to their different velocities. In contrast to the use of an acid pH (cacodylate buffer of pH 6.5) for the method of moving boundary electrophoresis the filter paper method of zone electrophoresis employs an alkaline buffer of pH 8.6 in which negatively charged protein particles move toward the positive electrode (the anode). With the contemporary interest in the relationship of abnormal hemoglobins to the hereditary hemolytic anemias paper electrophoresis has provided a readily accessible tool in the identification of the hemoglobins. This method incorporates certain basic features which can be briefly outlined.

PAPER METHOD In the paper method of electrophoresis the strip of paper is maintained in the horizontal position or is curved in the middle by suspension on one or more supporting rods. In either case the paper is saturated with buffer solution with the ends dipping into containers of the same solution. In the horizontal type of paper electrophoresis a length of filter paper wet with buffer solution rests on a plate of glass or plastic material with projecting points for support with the overlapping ends of the paper dipping vertically into troughs filled with the same solution. In adjacent compartments at each end are placed the respective electrodes. Before the solution is covered minute quantities of the red cell hemolyzate to be tested (prepared from the hemolysis of red cells in distilled water) are applied to the filter paper close to the cathodal end. In

the child and adult 2 per cent represents the upper limit of normal. In certain of the chronic hereditary hemolytic disorders fetal hemoglobin reappears in substantial amounts. Large quantities of fetal hemoglobin are synthesized in patients with sickle cell anemia (5 to 15 per cent), severe Cooley's anemia and to a lesser extent spherocytic anemia (usually less than 10 per cent).¹⁶⁴ The highest concentrations of alkali resistant hemoglobin are found in patients with severe Cooley's anemia in whom it may represent almost the entire hemoglobin content (12 to 100 per cent). The reason for these excessive amounts of embryonic hemoglobin is unknown and cannot be correlated with the severity of the disease. Since multiple transfusions tend to depress hemoglobin production, true estimates of its concentration can be made only during treatment free intervals or prior to the initiation of blood transfusions.¹⁷³

The presence of high concentrations of fetal hemoglobin in early infancy apparently suppresses formation of sickle hemoglobin¹⁶⁴ and hemoglobin C.¹⁷³ Sickling gradually increases after birth to reach maximal levels at 4 months of age. The replacement of fetal cells which are incapable of being sickled by red cells formed during the early months of life which are capable of being sickled is more closely correlated with the drop of fetal hemoglobin than with the absolute percentage of hemoglobin S.¹⁵⁷ This state of high level fetal hemoglobin and minimal sickle cell formation may serve as a protection against the probable dire effects of intracapillary sickling in utero where low oxygen tension prevails.

Fetal hemoglobin may be elevated in patients with pure red cell anemia (chronic congenital aregenerative anemia), acute and chronic leukemia and metastatic carcinoma involving the bone marrow. Normal values are present in patients with iron deficiency anemia and acquired hemolytic anemia.¹⁶⁴

Primitive Hemoglobin (P) Fetal hemoglobin is to be differentiated from the primitive type (P) which is found in early fetal life. This hemoglobin with an electrophoretic mobility on filter paper slower than those of the adult and fetal hemoglobins prevails during the first half of embryonic life and is later replaced by fetal hemoglobin. The primitive hemoglobin is associated with severe hypoproteinemia and agammaglobulinemia but its biologic significance awaits further study.⁷⁴

Hereditary Aspects One of the first steps in the diagnosis and appraisal of the degree of involvement in a patient with suspected hereditary hemoglobinopathy is the search for specific hemoglobins in the affected person and in other members of the family.

The principles relating to the familial distribution of the genes for the normal and abnormal hemoglobins conform to the basic principles of heredity. In the somatic cells of the body the genes are paired, one member of each pair being derived from one parent. The members of a pair of genes are termed alleles and occupy similar positions or loci on a pair of homologous chromosomes. The homozygous person receives an identical gene for the particular hemoglobin from both parents whereas the heterozygous person receives different contrasting or alternate members of genes or alleles. In the homozygous state both genes of the pair are either normal or abnormal and in the heterozygous individual one of the genes is normal and the other abnormal. In the case of abnormal hemo-

Type I is imperfectly separated from type A unless F appears in great excess. Hemoglobins S and D have identical mobilities. In its homozygous form type C migrates at a single focus between type S and I and is differentiated from the latter by its lack of resistance to alkali denaturation.¹⁰ The mobilities of types H and I appearing closest to the anodal end of the filter paper are greater than type A. The recently described hemoglobin J¹ possesses an electrophoretic mobility between hemoglobins I and A.

The relative mobility rates are summarized in Fig. 12 and are represented chemically in Fig. 13.

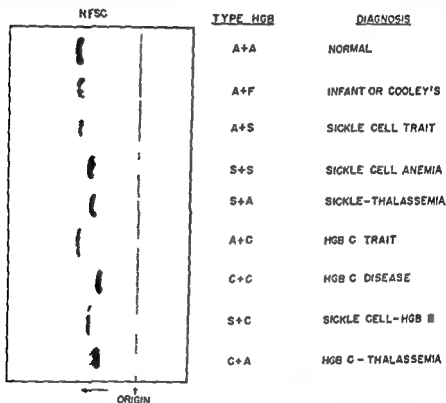


FIG. 13 Electrophoretic patterns of the common hereditary hemoglobinopathies (From Smith C H: *The Abnormal Hemoglobins: Clinical and Hematologic Aspects* J Pediatr 50:91, 1957.)

Fetal Hemoglobin. Important biochemical differences exist between adult hemoglobin and the form elaborated by the human fetus during prenatal life. Although the heme component of the two hemoglobin molecules is similar, the structure and properties of globin and possibly of the heme globin linkages are different. The property by which fetal hemoglobin is best known and most readily identified is its increased resistance to denaturation by alkaline solutions.

Fetal hemoglobin, which comprises 44 to 89 per cent of the hemoglobin in the newborn infant,^{11,2} decreases rapidly during the first year of life and is rarely demonstrable after the age of 30 months.¹ Following this period and in

Prior to the discovery of abnormal hemoglobins it had been established that the gene responsible for Cooley's anemia or thalassemia occurs once in the heterozygous or mild disease and twice in the homozygous or severe disease. A similar pattern of inheritance was postulated for sickle cell disease¹³¹—namely that the gene responsible for sickling is present in the heterozygous sickle cell anemia. Exceptions to this genetic pattern—namely the failure to find similar recognizable blood changes in each parent of an affected child with either Cooley's anemia or sickle anemia—can now be explained by the presence of another unrelated abnormal hemoglobin in the dissimilar parent.

Target Cells Target cells are particularly prominent in the blood smear of patients with abnormal hemoglobins and often provide a clue to diagnosis. In the Negro patient this distinctive feature is particularly suggestive of the presence of abnormal hemoglobins. Present in patients with both mild and severe Mediterranean anemia (usually up to 10 per cent) target cells are further increased in patients with sickle cell anemia and appear in even larger numbers in persons

Table 13 Common Abnormal Hemoglobin Syndromes

Type	Diagnostic Features of the Blood Smear	Percent Abnormal Hemoglobin
Sickle cell trait	Normocytic and normochromic red blood cells	24 to 45% Hb S
Sickle cell anemia	Normochromic or lightly hypochromic red blood cells anisocytosis poikilocytosis polychromasia target cells sickle cell occasional normoblasts	93 to 100% Hb S
Sickle cell-thalassemia disease	Large thin macrocytic cells marked anisocytosis basophilic stippling nucleated red blood cells moderate number of target cells occasional sickled cells	67 to 87% Hb S
Homozygous hemoglobin C disease	Slightly microcytic or normocytic normochromic red blood cells large number of target cells	100% Hb C
Hemoglobin C trait	Normocytic normochromic red blood cells target cells may be absent or increased	28 to 44% Hb C
Sickle cell hemoglobin C disease	Normochromic slightly microcytic red blood cells minimal anisocytosis and poikilocytosis numerous target cells	Hb S and Hb C each about 40 to 60%
Thalassemia hemoglobin C disease	Microcytic minimally hypochromic red blood cells marked anisocytosis and poikilocytosis with occasional cells basophilic stippling and micropherocytes	29 to 93% Hb C (usually 40 to 90%)

globins the double heterozygous state is one in which the genes determining the two hemoglobins differ from each other. In exceptional cases of thalassemia-sickle cell disease and of hemoglobin C-thalassemia other patterns of genetic transmission than multiple alleles are to be considered. These rare instances involve such chromosomal relationships as linkage in which genes are located in two different chromosomes permitting independent transmission.

Comprehensive family pedigrees will frequently elucidate not only the hematologic picture of the patient but also that of other closely related persons who are asymptomatic or who have suffered from a mild but unexplained anemia. Numerous family studies have demonstrated that with the exception of fetal hemoglobin the genes responsible for the abnormal hemoglobins are alleles of those for normal hemoglobin.

The gene for any one of the abnormal hemoglobins may thus replace one or both genes for normal hemoglobin. When only one normal gene is replaced the person is regarded as a carrier possessing a heterozygous hemoglobin trait. Homozygous or pure hemoglobin disease results when both genes for hemoglobin A are replaced by those of the same abnormal hemoglobin. With few exceptions family studies have demonstrated that hemoglobins S, C, D and E represent alleles of normal hemoglobin A.

Relation of Genetic Composition to Clinical Hematologic Variations. Expressivity of a gene refers to the variability of its expression in different persons and explains certain of the quantitative aspects observed in the inheritance of the abnormal hemoglobins. It has been postulated that the amount of a particular hemoglobin in a blood sample is influenced by interaction with the gene controlling another type of hemoglobin with which it is combined. Thus in a combination of Cooley's anemia and sickle cell disease the gene for Cooley's anemia permits the appearance of amounts of sickle cell hemoglobin as high as 50 per cent in the offspring, despite the presence in the parent of less than 50 per cent of this component. This augmenting influence which is also seen in the interaction of the gene for Cooley's anemia with the genes for either hemoglobin C and E will be elaborated later.

The intensity of each syndrome varies from the asymptomatic or mild case in which a single abnormal hemoglobin is present in the heterozygous state in combination with one of the two allelic genes for normal hemoglobin to the severe case in which two identical abnormal hemoglobins appear in the homozygous state.

Intermediate grades of hemolytic disease are associated with the presence of two different abnormal hemoglobins—the mixed or double heterozygous state. Their simultaneous presence results in a hemolytic anemia of variable intensity together with moderate splenomegaly and peripheral blood changes characteristic of both diseases. Although a single dose of a gene for abnormal hemoglobin produces the trait or mild disease and a double dose the severe disease the degree of clinical and hematologic changes cannot be exactly correlated with one particular mixture of abnormal hemoglobins. Thus Cooley's anemia and sickle cell anemia in both of which the homozygous state is represented are more severe clinically and hematologically than either homozygous hemoglobin C or E disease.

in the hemoglobin Pauling and associates¹¹ demonstrated that the electrophoretic mobility of sickle cell hemoglobin differed from normal hemoglobin. They also pointed out that these differences were based upon the number and kind of ionizable groups located in the globin fraction of hemoglobin rather than in the heme and that this molecular abnormality was responsible for the varied phenomena characterizing sickle cell anemia.

Hemoglobins are also identified by examining the digested fragments of the hemoglobin molecules on a chromatogram. Of the 300 amino acids forming the polypeptide chain of a half molecule of hemoglobin a significant difference is observed in sickle and other hemoglobins as compared with normal hemoglobin. Ingram¹² found that normal and sickle hemoglobin differ in one portion of their polypeptide chains. In each sickle peptide having nine amino acids valine replaces the normally occurring glutamic acid. This is presumably sufficient to alter the charge distribution on the surface of the molecule toward one favoring easy crystallization. Using the same method of trypsin and chymotrypsin digestion followed by "fingerprinting" of the peptide mixture Ingram¹³ also noted that the same glutamic acid of normal hemoglobin is replaced by the amino acid lysine in hemoglobin C disease. These findings with respect to both sickle cell hemoglobin and hemoglobin C reinforce the genetic evidence that these two mutations occur in similar places on the gene since they affect the same amino acid.¹⁴ According to this concept an altered gene is responsible for a corresponding alteration in the amino acid sequence of the protein with which it is identified.

The intrinsic features of the sickling mechanism becomes apparent when hemoglobin is reduced following exposure to low oxygen tensions or pH. The simplest method by which deoxygenation is accomplished is by placing a drop of blood under a sealed cover slip and observing the progressive increase in the number of sickle cells. Sickle hemoglobin is much less soluble than normal hemoglobin and even more so in the reduced state. Exposure to an environment of reduced oxygen tension causes sickle hemoglobin to become more viscous. This transformation is accompanied by intracellular crystallization¹⁵ and tactoid formation.¹⁶

Concentrated stroma free hemolysates prepared from the blood of patients with sickle cell anemia are extremely viscous when sickle hemoglobin is present in reduced states. Increases in viscosity of the whole blood accompany the multiplication of sickled forms and contribute to slowing of the blood flow. The tactoid form represents an altered and orderly alignment of the abnormal hemoglobin molecules in the anoxic state producing the sickle and crescent forms. The sickled erythrocyte has been described as a hemoglobin tactoid distorted by reticulated stroma.¹⁷ Crystallization accounts for the high concentration of hemoglobin in sickle cells attaining a mean corpuscular hemoglobin concentration of 50 to 60 per cent in the crystal as compared with values of approximately 34 per cent in the normal cell.

The amount of gelling of reduced hemoglobin involved in tactoid formation is influenced by the companion pigment particles which interact with it. Reduced hemoglobin must be sufficiently concentrated (above 10 per cent) for gelling and tactoid formation to occur. Actually it has been shown that a minimum of 7 per cent intracellular concentration of hemoglobin S pigment is needed to elicit the sickling phenomenon.¹⁸ With lesser amounts the erythrocyte may show a negative sickling test but sickle hemoglobin may be demonstrable by electrophoresis. The presence of hemoglobin A in the heterozygous person with the sickle

with the genes for both Cooley's anemia and sickle cell anemia (microcytic or thalassemia sickle cell disease) than in the person with only one of these genes for abnormal hemoglobin. In persons with sickle cell hemoglobin C disease they are present to the extent of 10 to 90 per cent of all red cells. It has been suggested that large numbers of target cells in the blood smear indicate the presence of hemoglobin C. Target cells are also increased in patients with hemoglobin E disease, perhaps less than in those with hemoglobin C disease. Few or none of these cells are noted in reported cases of sickle cell-hemoglobin D disease¹⁴ or of hemoglobins G, H, I, and J.

Syndromes Associated With the Abnormal Hemoglobins Investigation of the physicochemical properties of the hemoglobin molecule has been particularly revealing in explaining the variability in the clinical hematologic and genetic aspects of sickle cell disease and Cooley's anemia. These studies have demonstrated that each of these diseases exist not only as the classical homozygous forms with well known manifestations but also as variants. The latter though less severe nevertheless simulate sickle cell disease and Cooley's anemia clinically and hematologically. The differences can now be explained on the basis of the simultaneous presence of another hemoglobin. Thus the majority of abnormal syndromes to be described are initially diagnosed as either thalassemia or sickle cell disease.

The multiplicity of combinations of the abnormal hemoglobins in the heterozygous, double heterozygous, and homozygous states precludes a discussion confined exclusively to a single abnormal hemoglobin so that overlapping comments are inevitable.

SICKLE CELL DISEASE

Sickle cell disease is a comprehensive term used to include all those hereditary disorders whose clinical, hematologic, and pathologic features are related to the presence of sickle hemoglobin (hemoglobin S) in the red cells. Sickle cell disease is found primarily in Negroes. Relatively few cases have been reported in members of the white race. The majority of these cases occurred in persons of Italian, Greek, and Sicilian origin, suggesting an admixture of Negro blood with members of the Mediterranean races.^{6,7}

The sickle cell trait (sickleria) is present in those persons who are heterozygous for the sickling character and represents the combination of the sickle cell gene and the gene for normal hemoglobin. In persons with sickle cell anemia the gene for sickling is present in the homozygous state and results from the inheritance of sickling genes from both parents. The presence of a gene for another abnormal type of hemoglobin or the gene for thalassemia should be suspected in a child with sickle cell anemia when the blood of only one of the parents shows the sickle trait.

Sickling Phenomenon The sickling abnormality is attributed to a mutant gene which is responsible for the synthesis of a type of hemoglobin different from normal hemoglobin. The red cells that sickle are biconcave discs indistinguishable from the normal erythrocytes containing no sickle hemoglobin. Although it had been shown previously⁸ that the basic abnormality of the sickle red cell resided

Sickle Cell Trait

Incidence and Geographic Distribution The sickle cell trait representing the heterozygous state (hemoglobins A and S) occurs in approximately 7 to 9 per cent of American Negroes.^{1,9} A small number about 1 in 40 of those with sickling show sickle cell anemia. The highest frequency of the trait has been reported from East Africa where it varies from 30 to 45 per cent in some Bantu tribes. The peak of the incidence falls somewhat toward the west coast of Africa averaging 20 to 25 per cent on the coast.¹⁰ The high incidence of the sickle cell trait in several of the aborigine tribes of Southern India has suggested that the sickle cell trait may have been carried from this area to Africa.¹¹

Clinical and Laboratory Features The trait or sicklemia can be demonstrated *in vitro* by the ability of the erythrocytes to sickle when oxygen tension is lowered. This is accomplished by a variety of techniques such as sealing a drop of fresh blood under a cover slip with petrolatum or more rapidly by mixing the blood with a reducing agent such as 2 per cent sodium bisulfite. Sickling is slower in patients with the trait and the red cells assume a holly leaf appearance rather than the typical elongated pointed and filamentous form observed in patients with sickle cell anemia. The amount of sickle cell hemoglobin in the blood of persons with the trait as determined by electrophoresis varies from 24 to 45 per cent of the total hemoglobin.⁸ Target cells are present in small numbers but the red cells in stained blood smears are predominantly normal. The red cells of healthy persons with the trait have a normal life span.^{10,127}

Persons with the trait are not anemic, show no physical abnormalities and are usually asymptomatic. Splenic infarction resulting from vascular occlusion by sickled cells has been reported in patients with the sickle trait at moderate or high altitudes during flight.^{9,10,11,14} Incapability of concentrating urine normally,¹⁰⁴ gross hematuria,¹⁰⁵ and retinal hemorrhage¹⁰⁶ have also been described occasionally in patients with the trait.

Sickling and Malaria Since the observations by Beut¹⁶ that the sickle cell trait might protect the bearer from the effects of malaria many studies have been carried out to test this hypothesis. According to this concept the survival of persons with heterozygous sickle cell disease could offset the loss of persons with homozygous disease through malaria caused by *Plasmodium falciparum* but not other malarial parasites and thus maintain the high rates of sickling in malarious areas. Apparently sickle hemoglobin is unsuitable for growth of *P. falciparum*. Thus the loss of the sickle cell gene by early death of patients with homozygous sickle cell anemia is counterbalanced by the resistance of persons with the sickle cell trait to become infected with malaria. This concept of a selective or reproductive advantage for the person with heterozygous sickle cell disease over the normal person (balanced polymorphism) was also extended to thalassemia since many of the patients with this disease come from districts severely affected by malaria.¹⁸

More extended experience however based upon surveys in areas in which malaria and sickle cell disease prevail including direct inoculation of *P. falciparum* into persons with and without sickle cells has given conflicting evidence

cell trait decreases the amount of hemoglobin E required for gel formation and type C reduces this further. Sickling therefore depends not only upon the presence of hemoglobin S but also upon interaction with other hemoglobins. Type F hemoglobin present in the homozygous patient with sickle cell anemia exerts no significant influence on the gelling phenomenon.¹⁰

The sickling of the erythrocytes in patients with homozygous sickle cell anemia (SS) and other forms of sickle cell disease (thalassemia sickle cell and sickle cell hemoglobin C disease) is rapid and assumes chiefly a filamentous form. In persons with the sickle cell trait a holly leaf rather than the filamentous form predominates. The spicules of the holly leaf form represent the points of the tectoids.¹¹⁷

The sickle distortion is reversible so that the filamentous sickle cell which develops in an environment of lowered oxygen reverts to the normal discoid form when normal oxygen tension is restored. Oxygenation causes the crystallized hemoglobin S to go into solution again. Two types of sickle cells have been described: those reverting to the previously normal shape on exposure to oxygen and a smaller number which remain irreversible.¹⁸ Sickle cells appearing on the blood smears of patients with sickle cell anemia are of the irreversible variety. The blood smear of persons with the sickle trait is devoid of sickle cells.

Pathogenesis. The varied manifestations of sickle cell anemia result from the insolubility of reduced sickle hemoglobin and the formation of sickle cells which follows exposure to lowered oxygen tensions existing in the tissues. Although persons with the sickle cell trait heretofore had been regarded as uniformly asymptomatic it is now known that occasionally they too are susceptible to the same difficulties as the homozygous patient in situations in which there is reduced oxygen tension such as occur in airplane flights.¹¹⁴

The entanglement and enmeshing of rigid and inflexible sickle cells with one another increases the internal friction of the suspension resulting in an increased viscosity of the whole blood.⁸ The sickling and increased blood viscosity combine to produce capillary stasis, the formation of masses and plugs of impacted red cells, thrombi, hemorrhage, vascular occlusion, infarction and ischemic necrosis. Infection presumably increases plasma viscosity and results in an extreme degree of erythrocytosis.

The formation of sickle cells increases vulnerability to mechanical fragility, especially as the hemoglobin is deoxygenated.⁸ The shortened life span of the cells during movement in the circulation and passage through organs results in hemolytic anemia. Selective sequestration and stagnation of sickle cells in the spleen¹⁹ of the patient with sickle cell anemia probably results in their local destruction and for those cells that escape increased intravascular hemolysis. The cells from patients with the sickle cell trait and those from the patient with sickle cell anemia differ with regard to increased mechanical fragility. Mechanical fragility of the sickle cells of the patient with the trait begins to be affected at a level of deoxygenation at which the cells of the patient with the anemia already show a maximal increase. In general the *in vitro* behavior of sickle hemoglobin and sickle cells at reduced oxygen tensions are correlated with the severity of the clinical and hematologic features.¹

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Clinical and Laboratory Features The trait or sickle cell anemia can be demonstrated *in vitro* by the ability of the erythrocytes to sickle when oxygen tension is lowered. This is accomplished by a variety of techniques such as sealing a drop of fresh blood under a cover slip with petrolatum or more rapidly by mixing the blood with a reducing agent such as 2 per cent sodium bisulfite. Sickling is slower in patients with the trait, and the red cells assume a holly leaf appearance rather than the typical elongated, pointed and filamentous form observed in patients with sickle cell anemia. The amount of sickle cell hemoglobin in the blood of persons with the trait, as determined by electrophoresis varies from 24 to 45 per cent of the total hemoglobin.¹³ Target cells are present in small numbers but the red cells in stained blood smears are predominantly normal. The red cells of healthy persons with the trait have a normal life span.^{14,15}

Persons with the trait are not anemic, show no physical abnormalities and are usually asymptomatic. Splenic infarction resulting from vascular occlusion by sickled cells has been reported in patients with the sickle trait at moderate or high altitudes during flight.^{16,17,18} Incapability of erythrocytes to normally gross hematuria¹⁹ and retinal hemorrhages²⁰ have also been described occasionally in patients with the trait.

Sickling and Malaria Since the observations by Beut²¹ that the sickle cell trait might protect the bearer from the effects of malaria, a study has been carried out to test this hypothesis. According to this concept, the survival of persons with heterozygous sickle cell disease would offset the lethal potency of the malarial parasite through malaria caused by *Plasmodium falciparum* and not other malarial parasites and thus maintain the high rates of sickling in malarious areas. Apparently sickle hemoglobin is more suitable for growth of *P. falciparum*. Thus the loss of the sickle cell gene to individuals of groups with heterozygous sickle cell anemia is counterbalanced by the survival of persons with the sickle cell trait to become infected with *P. falciparum*. The concept of a selective or reproductive advantage for the person with heterozygous sickle cell disease over the normal person (homozygous normal) was also extended to the malaria resistance of the person with the sickle cell trait.²² It was concluded that the normal

individuals exposed to malaria become infected with malarial parasites and the normal individuals remain healthy. The heterozygous individuals with sickle cell trait are exposed to malaria but the sickle cell trait protects them from the effects of the parasite.

as to this protective relationship¹¹ There is greater unanimity with respect to children—namely those who are heterozygous for the sickling gene (hemoglobin A S) have a greater chance of surviving to reproductive age than do normal children when both are exposed to malarial infection¹¹

Although this subject is still controversial it appears that possession of the sickle trait affords some degree of protection against infection with *P. falciparum* The infection is of shorter duration and the parasite count is lower than in persons who do not possess the sickle cell trait⁹ Hence those who are heterozygous for the sickle cell gene appear to have a selective advantage in regions in which malaria is common

Sickle Cell (Drepanocytic) Anemia

Sickle cell anemia is a hereditary form of hemolytic disease occurring almost exclusively in Negroes and is due to the presence in the red cells of sickle hemoglobin the gene for which is present in the homozygous state Its incidence in American Negroes is 0.3 to 1.3 per cent¹² The disease is marked by anemia by painful and febrile episodes occasionally by aplastic crises and by a variety of manifestations due to distortion of the red cells The clinical syndrome was first described by Herrick in 1910³ in a Negro from the West Indies with chronic anemia in whose blood smear elongated and sickled cells were present The bulk of hemoglobin (60 to 98 per cent) is of the S variety with the remainder consisting of fetal (F) hemoglobin (usually 2 to 24 per cent) Normal (A) hemoglobin is absent unless the patient has had a transfusion recently

Pathology The basic changes in the tissues and organs are mainly due to a combination of capillary stasis obstruction by elongated and pointed sickle cells thrombosis of small vessels increased destruction of red cells and hemosiderosis The bone marrow is hyperplastic from excessive erythropoiesis and blood stasis¹¹ Of the nucleated cells in the marrow normoblasts predominate but more primitive forms of the red cell series are also present Only occasionally are sickled nucleated erythrocytes observed¹⁴ Leukocytosis is marked and megakaryocytes are increased Hyperplasia of the bone marrow is especially marked in children and accounts for the osteoporosis and subsequent expansion of the medullary spaces observed in the roentgenogram Fat embolism secondary to necrosis of the bone marrow has been described¹⁰³ Infarction of bone followed by embolization of bone marrow and fat to various organs is observed in patients with sickle cell-hemoglobin C disease as well as in those with sickle cell anemia¹ Focal infarction of the marrow contributes to the bone pain of a crisis Aseptic necrosis of the femoral head has been related to thrombosis of blood vessels supplying the affected area^{9,15}

Hemosiderosis from increased hemolysis of the patient's cells and occasionally from transfusions is especially pronounced in the liver bone marrow lymph nodes spleen and kidney

The spleen undergoes a series of changes progressing from congestive enlargement to fibrotic atrophy⁴ In younger people the spleen is slightly or moderately enlarged The splenic pulp is a deep red color due to intense congestion and engorgement with sickle cells the sinuses are compressed and infarcts are

common. Thickening of the capsule and fibrous tissue organization subsequently follows in areas of organizing hemorrhage. The siderofibrotic lesions in persons with sickle cell anemia resemble the siderotic nodules or "Gandy Camna bodies" of the spleen which occur in persons with spherocytic anemia and portal hypertension.⁴

In the final stages of the disease the spleen is a shriveled and scarred mass embedded in adhesions reflecting the replacement of the pulp by fibrous tissue. Atrophy from this sequence occasionally results in extremely small spleens (in splenectomy). The size of the spleen therefore decreases as its vascularity diminishes as the hemorrhages and infarcts become organized and as fibrous tissue replaces the residual pulp.

The liver is often markedly altered both in structure and in function. Liver dysfunction and necrosis are brought about by severe impairment of hepatic flow due to the combined effects of anemia and capillary obstruction by masses of sickle cells and Kupffer cells distended with phagocytosed red cells. Focal necrosis, loss of liver cells, scarring and cirrhosis characterize liver involvement.⁶ Cholelithiasis due to increased destruction of red cells is not infrequently observed in patients with sickle cell anemia.¹⁰

The kidney lesions in patients with severe sickle cell anemia are marked by congestion of glomerular capillaries and of tubular arterioles with sickle cells and by hemosiderosis and scarring. Hematuria is ascribed to congestion of small vessels, thrombosis, stasis and infarction, necrosis.⁴ Changes in the central nervous system are primarily intravascular mainly due to thrombosis of small vessels in the meninges and cerebral cortex resulting in hemorrhagic degenerative and atrophic changes.⁹ Obliterative endarteritis of the cerebral vessels may also contribute to the causation of symptoms.¹¹ Chronic leg ulcers occur in adolescents and adults frequently in the lower third of the leg on the inner side just above the ankle.¹² They are usually punched out in appearance, single or multiple and unilateral or bilateral. The histologic picture is that of chronic granulosomatous ulcer. There is no evidence that ulceration originates from thromboses due to the blocking of capillaries by sickled erythrocytes.³

Clinical Features: Sickle cell anemia is usually recognized during the preschool period. In one half of the patients symptoms are present by 2 years of age,¹ although clinical manifestations are rare in the first six months of life. In a few recorded cases the disease has been diagnosed between 1 and 3 months of age.¹³ In one series¹⁴ the average age at onset of symptoms necessitating hospitalization ranged from 3 months to 15 years.

As already pointed out excessive blood destruction and the pathologic tendency toward intravascular sickling, stasis and thrombosis in various organs and tissue sites are responsible for the multiplicity of symptoms and the confusion with the other clinical syndromes. In children the most frequent complaints are joint back and abdominal pain, vomiting, fever, frequent upper respiratory infections, fatigability and anorexia. Other less commonly encountered symptoms are headache, meningismus, dyspnea, weakness, epistaxis (without purpura), convulsions, lethargy and listlessness. Episodes of acute abdominal pain may be severe, accompanied by fever, muscle spasm, nausea, vomiting.

and leukocytes.¹¹⁸ These abdominal crises may have a fatal outcome and have been attributed to shock from massive sequestration of sickled red cells, plasma loss and circulatory failure.¹⁰¹

Central nervous system manifestations may be the initial complaints or may occur later in the disease. Pain in the extremities may be muscular or be based upon bone and joint involvement and often simulates osteomyelitis. Arthralgia may be severe. Inflamed and swollen joints occur but are infrequent. Localized swellings presumably due to erythrostasis with sickle cells develop over bony structures such as the jaw and long bones. Exacerbations of these complaints occur periodically and are often related to upper respiratory infections. In infants under 2 years of age dactylitis with marked pain and swelling of the hands and feet are occasionally observed (hand foot syndrome). It has been stated that swelling of the hands and feet in a Negro infant in whom there is no readily apparent cause for the swelling warrants a suspicion of sickle cell anemia.¹

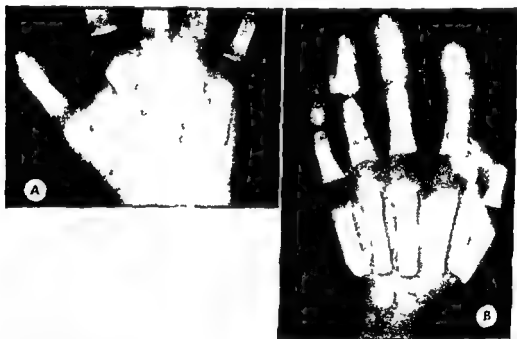


Fig. 14 Hand foot syndrome in infants with sickle cell anemia. A Note evidence of subperiosteal bone formation and area of rarefaction in the body of the third metacarpal. B Note complete regression after one month; these lesions are transient.

Abnormal roentgenologic findings do not appear for the first week or ten days and are confined largely to the metacarpals and metatarsals and extend infrequently to the phalanges. Abnormalities consist of blurring of the margins and a variable degree of rarefaction within the medullary cavities. Spontaneous regression occurs without residual signs.¹⁹

Physical findings vary considerably and include marked pallor of the mucous membranes, greenish yellow discoloration of the sclerae, lymphadenopathy, etc.

diac enlargement and splenomegaly. Liver enlargement may be slight to moderate. The spleen often slightly to markedly enlarged in children atrophies with advancing age due to infarction and fibrosis. The incidence of splenomegaly has been found to be 33 per cent in the first decade of life and 10 per cent thereafter.¹⁰⁷ Although splenomegaly is not uncommon in patients with sickle cell anemia it appears to be much more frequent in those with sickle cell-hemoglobin C disease and sickle cell thalassemia disease. An enlarged spleen is often associated with shortened survival of transfused normal cells.¹¹⁶ Less common are ascites, joint swelling, osteomyelitis, gallstones, and hemiplegia in patients with severe nervous system involvement. The veins of the retina may be dilated and tortuous; the arteries show this feature to a lesser extent.^{118a} Retinal hemorrhage has been observed.⁸¹ Enlargement of the heart and the occurrence of apical systolic murmurs are indicative of hypertrophy and dilatation in response to prolonged anemia and anoxia.¹¹⁶ Pericarditis is also noted. Episodes of bronchopneumonia occur commonly and are attributable both to infection and to pulmonary infarction. Cor pulmonale in older patients may be the consequence of multiple recurrent infarctions.¹²

The inability to concentrate urine normally is a feature of sickle cell anemia and to a lesser extent the sickle cell trait.^{71, 110} In patients with sickle cell anemia the concentration defect is corrected by transfusion of normal red cells which are known to suppress intravascular sickling.^{146, 147} Children with sickle cell anemia are predisposed to salmonella osteomyelitis.^{6, 7} Aseptic necrosis of the capital epiphysis of the femur and less commonly of the humerus occurs especially in older children and adults with homozygous sickle cell disease.^{70, 111} sickle cell-hemoglobin C disease and sickle cell thalassemia. The osseous lesions probably result from thromboses of the blood vessels supplying the affected areas, presumably due to the sickling phenomenon. Involvement of the femoral neck and head produces the clinical and pathologic picture of classical Legg-Perthes disease. Abnormal electroencephalograms are frequently observed in patients with sickle cell anemia and probably reflect blocking of the blood supply to various parts of the brain with varying damage to nerve cells.¹ In contrast to its frequent presence in adults, chronic leg ulceration is rare in children.

The typical linear habitus with narrow hips and shoulders, decreased stature, increased upper dorsal kyphosis and lumbar lordosis, increased anteroposterior diameter of the chest, and hypogonadism are common in the adult patient. Many of these changes are also present in children, although to a lesser extent, unless the disease is severe in early life. Children often have a barrel-shaped chest, an enlarged and protruding abdomen, and thin extremities.¹

Skeletal Changes. The skeletal abnormalities as revealed by roentgenographic examination are similar to those seen in patients with severe Cooley's anemia but are less marked and less common.¹²⁷ They are based upon bone marrow overgrowth resulting in osteoporosis, widening of medullary spaces, and thinning of the cortices. These alterations are observed in the skull, vertebrae, long bones, hands, and feet. The skull changes consist of widening of the diploic spaces, external displacement of the outer table, and a hair-on-end appearance produced by the radial arrangement of the bone trabeculae so common in patients with

Cooley's anemia. Skeletal changes are more frequent in adolescents and adults with sickle cell anemia than in children. In adult life the hematopoietic tissue is replaced by connective and osseous tissue. New bone formation on the inner aspect of the cortex accounts for the thickening of the cortex and narrowing of the medullary cavity.¹⁷ Osteoporosis, destruction and collapse of the weakened vertebral bodies, especially in the lower thoracic and lumbar regions, cause compression deformities in both children and adults.¹⁸

Blood. The anemia is chronic normochromic and normocytic with signs of increased regenerative activity of the bone marrow. The blood smear shows round and oval cells, anisocytosis and poikilocytosis. Hyperchromia and polychromatophilia are less common. Microcytosis appears when the anemia is severe. Sickled cells vary in number and are often surprisingly few in patients with severe disease. One of the standard deoxygenation techniques shows sickling to be complete and rapid in all red cells in patients with sickle cell anemia in contrast with slow development in the red cells of those with the sickle cell trait. Sickle cells (drepanocytes) are crescent or holly leaf shaped in appearance in persons with the sickle cell variants but in those with the homozygous disease they are characteristically elongated filamentous pointed at each end curved in the middle and contain a full complement of hemoglobin. Target cells are increased in number. Nucleated red cells, chiefly normoblasts, vary from a few to many, especially in patients with pronounced anemia and occur in the presence of pneumonia or other severe infections. Osmotic fragility is decreased, mechanical fragility increased,¹⁹ and red cells have a shortened survival time.^{10, 16}

Bilirubin ranges from 1 to 3 mg per 100 ml, with the major portion of the indirect type. The number of reticulocytes is increased, ranging from 5 to 25 per cent. Excessive reticulocytosis indicates a superimposed acute infectious process or an immune hemolytic anemia. In one 18-month-old infant with a herpetic lesion, an added acquired hemolytic anemia was evidenced by a hemoglobin level of 4.6 gm per 100 ml, reticulocytes of 78 per cent, and a transient positive Coombs test.¹⁰⁰

Patients with sickle cell anemia maintain a relatively stable hemoglobin concentration with equilibrium between blood destruction and production established between 6 and 9 gm per 100 ml without transfusions. This stability is explained by the increased effective erythropoiesis in patients with this disease despite increased rates of destruction. By contrast in patient with thalassemia major there is less blood destruction but also less effective production—hence the need for multiple transfusions.¹⁴ The red cells range between 2 and 3.5 million per cubic millimeter. With an infection or aplastic crisis the hemoglobin may drop below 5 gm per 100 ml and the red blood count below 2 million per cubic millimeter.

The leukocytes range between 10,000 and 20,000 per cubic millimeter. A count of 30,000 or more occurs in patients with complications such as pneumonia and are associated with a marked shift to the left of polymorphonuclear neutrophils and the appearance of myelocytes. The platelet count is normal or slightly elevated. The sedimentation rate¹ is usually slow even in the presence of marked anemia because of the abnormal shape of the sickle cells and poor rouleaux formation.



Fig 15 A and B Stained blood smears of patient with homozygous sickle cell anemia. Note elongated and narrow sickle cells with pointed ends. The nonsclered red cells vary in size and are round or oval. A: Courtesy Dr. Ralph L. Engle, Jr., New York, N. Y. B: courtesy Dr. Ralph L. Engle, Jr., New York, N. Y., and Dr. David Lawrence, Lakeville, Conn.

Crises The symptomatic or clinical aspects of the crises in patients with sickle cell anemia are discussed separately from the characteristics of the aplastic and hemolytic crises in these patients.

Symptomatic or Clinical Crises With rare exceptions a crisis is characterized by fever and severe pain in the joints, back, and extremities, usually without exacerbation of the anemia. At times these episodes are accompanied by a slight rise in hemoglobin concentration. The number of reticulocytes, serum bilirubin concentration, and excretion of urobilinogen in the urine and stool are not increased.

This is the common type of crisis in patients with sickle cell anemia and is designated as the clinical and symptomatic type.¹¹ There is suggestive evidence that this type of crisis in childhood is associated with a sharp shrinkage of the plasma volume so that the peripheral hemoglobin concentration may actually be elevated. Furthermore, the anemic patient with this disease has a blood volume characteristically far in excess of the normal in periods of freedom from disability.^{12,13} As would be expected in restoring expanded plasma levels during treatment with whole blood, plasma or dextran, the hemoglobin level may actually drop. Sudden death occurring in infants and children during a severe crisis in sickle cell anemia has been reported.¹⁴ The most striking necropsy finding in these patients was congestion of the internal organs with sickled erythrocytes.

Aplastic and Hemolytic Crises The existence of a hemolytic crisis accompanied by a sudden destruction of blood and hyperfunctioning bone marrow in patients with sickle cell anemia has been challenged.¹¹ (p. 217) The aplastic or regenerative crisis on the other hand is well documented. It is characterized by a rapidly developing anemia due to the cessation of red cell production and the continued destruction of sickled cells in the circulation. However, hemolytic crises in patients with sickle cell anemia have occasionally been described in conjunction with an associated infection such as pneumonia or septicemia and are accompanied by severe pain in the back, abdomen and extremities. A significant drop in red cells and hemoglobin and an increase in both the indirect serum bilirubin and reticulocyte count accompany these symptoms.¹⁵

Nutritional megaloblastic anemia has been reported in patients with sickle cell anemia and sickle cell-hemoglobin C disease as in patients with other congenital hemolytic syndromes.¹⁶ It has been attributed to an excessive need for folic acid, vitamin B₁₂ or both during periods of active growth and in the presence of pronounced erythropoiesis. A reversal to normoblastic erythropoiesis follows specific therapy. Transient episodes of megaloblastosis of the bone marrow occurred in a patient with sickle cell anemia complicated by an extracorporeal hemolytic state.¹⁷ In this patient a relative deficiency of hemopoietic factors could have resulted from the severe hemolytic process.

Diagnosis The demonstration of the sickling phenomenon *in vitro* prevents mistakes in diagnosis. The basic pathologic process accounts for diverse symptom complexes which simulate rheumatic fever, osteomyelitis, polyomyelitis, encephalitis,^{1,8} and other neurologic disorders and appendicitis and other acute surgical emergencies.

Although sickle cell anemia mimics rheumatic fever especially when associated with joint pains, fever and leukocytosis, the coexistence of the two diseases is rare.^{1,18} Episodes in which the patient appears critically ill with abdominal pain, nausea and vomiting may be severe and recurrent and extremely difficult to differentiate from a surgical condition requiring a laparotomy. The utmost conservatism must be exercised in management when it is established that the patient has sickle cell anemia. It is entirely possible, however, for the patient to suffer from the same surgical emergencies as persons without sickle cells. The combination of severe bone pain and fever requires differentiation from osteomyelitis. Except for the infrequent complications of salmonella osteomyelitis

and aseptic necrosis of the femoral and humeral capital epiphyses osseous involvement requiring treatment is uncommon

Treatment Since there is no specific therapy symptomatic and supportive measures are mainly relied upon¹¹¹ The treatment of the patient with the painful or clinical crisis in whom anemia is not a major factor demands the most serious consideration Bed rest aspirin codeine or other analgesic and sedative drugs usually suffice to control symptoms in the average case Oral hydration intravenous infusions of glucose or dilute electrolyte solutions increases blood volume and expedites mobilization of stagnant or tripped sickle cells¹¹² Antibiotics are prescribed if infection is present Hemodilution tends to overcome the shrinking of plasma volume with which the crisis is associated and to restore it to precrisis levels The excessive plasma volume noted in patients who are not in crisis observed in a small number of crises¹¹³ emphasizes the need for fluids Plasma dextran and other plasma extenders⁶⁰⁹ are useful in achieving this aim

In contrast to patients with severe Cooley's anemia whose blood levels decline progressively unless supported by transfusions are patients with uncomplicated sickle cell anemia whose hemoglobin levels tend to stabilize between 6 and 9 gm per 100 ml without transfusions Transfusions of packed cells are given for correction of severe anemia accompanying infection for aplastic crisis and for relief of extreme pain when other measures fail Transfusions raise hemoglobin levels increase the oxygen capacity of blood and reduce the number and concentration of sickle cells both by dilution and by the depressing action of blood supplements on hematopoiesis^{140 111 113}

Oxygen therapy is useful in combating severe anoxemia especially in patients with congestive heart failure but should only be employed for short periods of time Continuous treatment with oxygen depresses bone marrow and aggravates the anemia¹¹³ A variety of agents each designed to relieve the painful crisis through individual mechanisms have been employed These include Priscoline¹¹⁴ ACTH and cortisone¹¹⁵ and intravenously administered sodium carbonate⁶ More extended trial is required with each of these methods of treatment before a definite opinion can be given as to their merit Cobaltous chloride may produce initial satisfactory hemotopoietic responses but these are not sustained The serious disadvantage of its use in addition to anorexia nausea and vomiting is the depression of thyroid function

Splenectomy in the treatment of patients with sickle cell anemia has had its advocates¹¹⁶ but it does not occupy the therapeutic position that it does in patients with spherocytic anemia and to a lesser extent Cooley's anemia In one series of patients (Dickstein and Koop quoted by Margolis¹¹⁶) with marked to moderate enlargement of the spleen no major crises followed removal of the spleen Since an enlarged spleen constitutes the main site of sickling sequestration and destruction of the sickled cells¹⁰ benefits from splenectomy should be substantial Furthermore removal of a large spleen increases the average erythrocyte survival time of the patient's red cells by one third (a half life of 37 days of Cr⁵¹ presplenectomy is compared with a half life of 11.4 days postsplenectomy)¹¹⁷ Nevertheless because of the natural tendency for the spleen to shrink in size and to atrophy its removal is not advocated as a routine measure It should

be reserved for the occasional patient with a very large spleen who requires excessive transfusions. In such patients normal red cells are presumably destroyed by the development of an extracorporeal hemolytic defect.¹¹⁰

There is no contraindication to tonsillectomy when attacks of acute tonsillitis are frequent and associated with respiratory infections and a crisis. Preoperative transfusions and other safeguards are necessary.¹¹¹

Prognosis. The prognosis is very unfavorable with a fatal outcome in many patients in childhood or early adult life. Death results from overwhelming infection, congestive cardiac failure, cerebral vascular accidents, and abdominal crises.¹¹¹ With the use of antibiotics, chemotherapy, transfusions, and oxygen in selected patients, life expectancy can be prolonged. The outlook for the patient with the variants of sickle cell disease is more favorable than for those with homozygous sickle cell anemia.¹¹²

THALASSEMIA (COOLEY'S ANEMIA, MEDITERRANEAN ANEMIA, ERYTHROBLASTIC ANEMIA, HEREDITARY LEPTOCYTOSIS)

Thalassemia is a hereditary hemolytic disorder occurring predominantly in persons of Mediterranean origin and is characterized by abnormalities of red cell structure and hemoglobin synthesis resulting from a defect in erythropoiesis. The disorder ranges in severity from the asymptomatic trait to the severe form requiring frequent transfusions. In its severe form it combines the features of a refractory anemia with typical roentgenographic, clinical, and hematologic features.

Historical. In 1925 and in 1927 Cooley and associates¹ drew attention to an anemia which had hitherto been regarded as belonging to the heterogeneous group of von Jaksch's anemia but which possessed such well defined features as to constitute a definite clinical entity. Outstanding features were its anemia, icterus, familial aspects, characteristic facies, skeletal changes, splenomegaly, and the appearance of large numbers of circulating normoblasts in the peripheral blood. Because of the latter aspect the disease was initially termed erythroblastic anemia. From studies of the families of patients with the severe disease it soon became apparent that the siblings and parents possessed the features of the disease in mild form.

Nomenclature. The designation of thalassemia for Mediterranean or Cooley's anemia has shown increasing usage with the recognition of the abnormal hemoglobins. Although there may be some reservations with regard to its derivation,² it lends itself to the terminology indicating combination with various abnormal hemoglobins and to qualification as to the degree of severity.

Race and Incidence. The majority of patients have a Mediterranean ancestry, most commonly Italian, Syrian, and Greek. In Italy the highest prevalence is in the lower Po valley in northeastern Italy around Ferrara³ and in southern Italy. The Italian islands of Sicily and Sardinia, the island of Corsica, and the Greek islands of Cyprus and Crete have large concentrations of affected persons, probably due to inbreeding.⁴ The disease has been reported in a variety of non-Mediterranean races with wide geographic distribution. It has been found among the

Chinese (spreading from Canton and Hongkong) and other Orientals Asiatic Indians Egyptians Britons Germans Bukharan and Kurdistan Jews¹¹⁹ Negroes⁴ and Mexicans to mention only a few.¹⁶¹ In some areas of Thailand¹¹¹ the incidence is high and represents one of the most common forms of hemolytic anemia. Its spread from areas adjoining the Mediterranean Sea by Greek traders in commerce colonizations and explorations may account for the present day frequency with which this disease is found in southern Asia.¹⁰⁰ It would seem that mass migrations carried the genetic defect eastward to China from a single focus in the northern Mediterranean.⁹ There is still the possibility, however, that spontaneous mutations arising in a number of areas account for the widespread nature of the thalassemia gene. If the frequency with which the mild type of Cooley's anemia is encountered in New York City is an indication of its extent among persons of Mediterranean origin in other parts of the United States, this condition is extremely prevalent. In New York City persons with the mild or severe disease are already separated by several generations from those originally born in southern Italy and Greece. Neel and Valentine¹² on the basis of known incidence of the severe form computed the mild form as occurring in approximately 4 per cent of adult Italians in the city of Rochester, N.Y. The bulk of Italians immigrated to the United States from the southern part of their country in the latter half of the nineteenth century and the beginning of the twentieth.⁶ It is in the offspring of this group in New York City that the largest number of cases of Cooley's anemia occur.

Genetic Transmission The person with the trait or mild disease who functions as a carrier is heterozygous for a gene which when homozygous produces the severe disease. The gene for thalassemia has a varying expressivity for the grade of anemia and sometimes it is difficult to separate the moderate from the severe type hematologically. The presence of the trait in only one parent of a child with the severe disease should prompt a search for the sickle cell trait, hemoglobin C disease or hemoglobin E disease in the other parent and for the combined disease with the different hemoglobins in the patient.

Effect of the Gene for Thalassemia Upon Other Hemoglobins The gene for thalassemia either interferes with the normal suppression of fetal hemoglobin or the latter is formed in place of the more difficult synthesis of the adult type of pigment. The presence of excessive amounts of hemoglobin S, C and E in combination with thalassemia may reflect a compensatory replacement of normal hemoglobin whose synthesis is retarded by the gene for thalassemia. As already has been postulated, these increased concentrations may also reflect the potentiating effect of the gene for thalassemia on the formation of abnormal hemoglobin. Exception to this principle has been found in connection with a thalassemia gene associated with a low A hemoglobin percentage which fails to interact with the genes for hemoglobin S, C and E. This contrasts with the more common interacting thalassemia gene which is responsible for an elevated A component.⁹ (p. 276)

Clinical Types The disease presents a wide and continuous spectrum of severity designated at each extreme as thalassemia major for the severe type and thalassemia minor for the mild type. In the affected person these correspond to

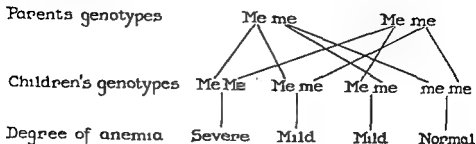


Fig 16

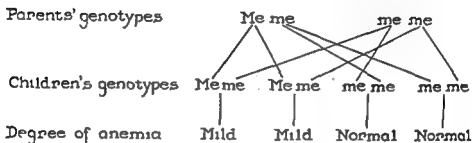


Fig 17

Figs. 16 and 17 Schematic representation of genetic transmission in Cooley's anemia. Me is a gene for Mediterranean anemia (Cooley's anemia thalassemia) me is the contrasting normal allele. The designation of $Me me$ is the heterozygote, a simplification of current terminology for the trait or mild form of this disease. $Me Me$ is the homozygote (severe disease thalassemia major) $me me$ is the normal individual.

Fig. 16 Inheritance of the severe type of Mediterranean anemia when both parents are heterozygous. (From Smith C. H. *The Abnormal Hemoglobins Clinical and Hematologic Aspects* J. Pediat. 50:91, 1957.)

Fig. 17 Inheritance of the mild type of Mediterranean anemia when one parent is heterozygous and the other normal. (From Smith C. H. *The Abnormal Hemoglobins Clinical and Hematologic Aspects* J. Pediat. 50:91, 1957.)

the homozygous and heterozygous states respectively. In both the major and minor types there are also varying grades of severity. Thus thalassemia minor embraces forms which are asymptomatic without anemia (termed the trait or thalassemia minima) and the minor type per se which is usually associated with mild to moderate anemia. Patients with thalassemia minor or minima being heterozygotes remain true to type and never develop the severe disease. Italian writers refer to Rietti-Greppi-Micheli's disease as a heterozygous type much less severe than thalassemia major and described by them as hemolytic icterus with increased red cell osmotic resistance to fragility.^{1,2}

The homozygous disease in which each parent is heterozygous for thalassemia falls into two classifications on the basis of grade of severity—the intermediate (moderate) type in which the patients function effectively without transfusions and the severe type in which the patients require multiple blood supplements to maintain hemoglobin levels compatible with normal activity. In contrast to

the severely affected patients those in the intermediate group are able to maintain concentrations of hemoglobin ranging from 7 to 10 gm per 100 ml without treatment. On the other hand patients with the intermediate type are observed in whom the disease is quiescent for many years until quite suddenly transfusions are required. This occurs following infections or exposure to noxious agents or for no apparent reason. From this point on the need for transfusions may become so urgent that splenectomy is necessary.

Fetal Hemoglobin in Patients With Thalassaemia In patients with the severe disease an extraordinary percentage of the circulating hemoglobin is of the alkali resistant or fetal type with values ranging from 40 to 100 per cent.^{11, 14, 16} In parents and asymptomatic siblings with the trait of the disease fetal hemoglobin lies within the normal range (less than 2 per cent).

Except for the marked elevation of fetal hemoglobin no specific abnormal hemoglobin has been described in patients with this disease. No relationship has been demonstrated between the amount of fetal hemoglobin in an individual patient and the severity of the disease. It has been suggested that the increase in fetal hemoglobin stems from interference by the gene for thalassaemia with the synthesis of adult hemoglobin so that reversion to fetal hemoglobin formation results. In several infants 1 and 2 months of age in whom clinical and hematologic features were already indicative of thalassaemia major fetal hemoglobin exceeded the elevated levels normally found at this age.¹ These observations indicate that the high level of fetal hemoglobin synthesis is a continuous process from fetal to adult life and that interference with the formation of normal adult hemoglobin is a permanent feature of the severe disease.

With overnight electrophoresis fetal hemoglobin when present in substantial amounts can be demonstrated as a slower component than normal hemoglobin and almost continuous with it.

Pathogenesis Defective synthesis of normal hemoglobin with an increased percentage of fetal hemoglobin, impaired fabrication of circulating erythrocytes¹⁴ and increased hemolysis of the defective cells have been implicated in the causation of the disease. More specifically there is evidence that heme synthesis may be impaired by a relatively slow rate of protoporphyrin synthesis and by a partial block in the combination of protoporphyrin and iron. Globin formation remains unaltered. It has been demonstrated in patients with severe Cooley's anemia that the problem of establishing equilibrium compatible with normal activity is greatly exaggerated by the inability of erythropoiesis to compensate for the accelerated rate of red cell destruction and this inadequacy is greater than that in patients with other hemolytic anemias. This accounts for the greater need for transfusion in patients with thalassaemia than in those with sickle cell anemia in whom production is more effective.

Pathology The anatomic changes reflect the abnormalities that characterize the disease—namely increased destruction of structurally defective red cells, increased regeneration with erythropoietic hyperplasia of the bone marrow, extramedullary hematopoiesis, deposition of excessive iron in tissues resulting from hemolysis of transfused red cells and the patient's own red cells and the long term effect of chronic hypoxia.

The skeletal structures contain abundant brown red and chocolate colored marrow the bony trabeculae are thinned and often the site of new bone formation. The spleen is firm hard and greatly enlarged. The pulp is abundant cellular and fibrotic tissue is increased. Extensive hematopoiesis is noted chiefly of the red cell series and to a lesser extent of other myeloid elements. The follicles are atrophied.¹⁸

In some patients visceral siderosis and fibrosis of the liver and pancreas are especially marked. The liver parenchyma is divided by broad irregular bands of fibrous tissue into nodules of varying sizes. Extreme hemosiderosis is diffuse throughout the liver so that iron pigment often fills the cytoplasm of the cells. Hepatic cells regenerate and show much less pigmentation. In the pancreas extensive and diffuse interlobular and intralobular fibrosis is frequently observed with a similar division of the parenchyma into nodules. The anatomic changes in the liver and pancreas are frequently identical with those characterizing idiopathic hemochromatosis.¹ Lymph nodes show pigmentation and are also the site of increased erythropoiesis.

Hemosiderin deposits are especially marked in the liver spleen and pancreas and are also found in the thyroid parathyroid and adrenal glands distal convoluted tubules of the kidneys bronchial glands gonads lymph nodes lungs and myocardium.¹⁹ The heart is dilated and hypertrophied showing dense hemosiderotic staining of the myocardium interstitial fibrosis and hypertrophy of the myocardial fibers.

Clinical Features The clinical features of thalassemia major and thalassemia minor are discussed separately.

Thalassemia Major Thalassemia major begins insidiously in infancy and is sufficiently developed in most patients to be recognized clinically in the latter half of the first year of life. Pallor diarrhea fever poor feeding and an enlarged spleen in children of Mediterranean origin lead to suspicions of the diagnosis. The severely anemic patient presents a pathognomonic facial appearance including prominent frontal and parietal bosses enlargement of the head prominent malar eminences depression of the bridge of the nose puffy eyelids a mongoloid slant of the eyes often with an epicanthal fold enlargement of the superior maxilla exposing the upper teeth a muddy yellow complexion and icteric tint to the conjunctivae. Other characteristic features are small stature and hypogonadism. Hypertrophy of the upper maxilla may lead to eventual marked malocclusion especially involving the frontal central incisors.¹⁹ (See Figs 18 and 19.)

Lymphadenopathy is occasionally noted. The abdomen protrudes when splenomegaly becomes prominent especially in the younger child. Splenomegaly is progressive and abdominal enlargement becomes disabling. Initially the liver is enlarged to a lesser degree but becomes markedly enlarged especially after splenectomy. Gallstones^{1, 6} and chronic leg ulcers^{21, 24, 111} are rare occurrences. nosebleeds are more frequent. Pathologic fracture of the femur is an infrequent complication and is due to cortical atrophy. With prolonged and intensive transfusion therapy the clinical evidence of hemosiderosis is superimposed on the manifestations of chronic anemia. The patient with Cooley's anemia who has had fre-



Fig. 18 Characteristic facies in severe Cooley's anemia (thalassemia major). Note prominent malar eminences, depression of the bridge of the nose, slight oblique appearance of eyes, and enlargement of superior maxilla with protrusion of lip upward exposing upper teeth.

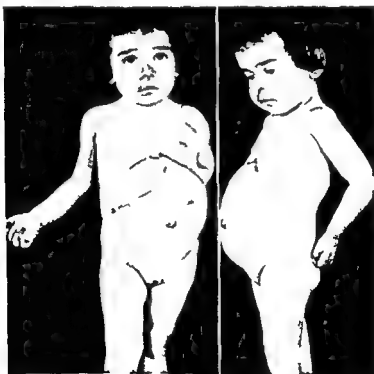


Fig. 19 Severe Cooley's anemia showing enlargement of liver and spleen and protrusion of abdomen. Facies are similar to those of patient shown in Fig. 18.

quent transfusions shows a deep brown pigmentation and darkening of the skin due to increased amounts of melanin in the epidermis and hemosiderin in the dermis.¹¹ The skin gives a tanned bronzed coloration with fine dark stippled like freckles.

Cardiac dilatation and enlargement and **hemic murmurs** are common. Asymptomatic cardiac enlargement begins in late childhood and slowly increases. Pericarditis of the acute benign nonspecific type has been described in patients with severe Cooley's anemia especially those who have had splenectomy.¹ In all of these patients the pericarditis was self limited and ended in complete recovery. The basis for this peculiar susceptibility is unknown. Children with Cooley's anemia whose hemoglobin levels are markedly lowered undergo prolonged effort with surprisingly little disability. Headache precordial and bone pain listlessness and anorexia appear when the hemoglobin levels drop below 7 gm per 100 ml and are relieved by transfusions.

Thalassemia Minor The patient with the trait is asymptomatic and the disease goes unnoticed usually being detected in the course of a family survey when a sibling is suspected of having the severe disease. Persons with the trait or mild type do not show the cardiac murmur enlargement of the heart or bone and joint pain of the severe type. Patients with mild or moderate anemia are often treated for iron deficiency anemia until the lack of response to therapy prompts a blood examination and the true diagnosis is disclosed. Those with the mild disease show no facial abnormalities or skeletal changes. In persons with the moderate or mild disease the spleen may be slightly enlarged or not enlarged at all.

Growth and Maturation In patients with thalassemia minor growth is normal. In some patients growth and maturation of the skeleton are retarded and Caffey¹⁴ regards patients with Cooley's anemia as the best examples of skeletal dwarfism and infantilism caused by chronic anemia. Children with the severe disease grow normally until about the age of 8 to 10 years at which time their growth rate undergoes marked retardation so that they attain a very short final height. Secondary sexual characteristics develop later than in the normal population. Normal menses are rare and cease several months to several years after the onset.

Skeletal Changes The skeletal changes as revealed by roentgenographic examination in persons with Cooley's anemia reflect the overactivity and overgrowth of the bone marrow.¹ Extreme marrow hyperplasia results in osteoporosis widening of the medullary spaces thinning of the cortex trabecular atrophy coarse reticulation with regeneration of new bone which is a later development in the disease and thickening of the skull. The frontal bone is the site of early and marked thickening. Overgrowth of the marrow in the paranasal sinuses and mastoids interferes with their pneumatization and occasionally completely supresses it.¹⁸ Lateral views of the skull show an enlarged diploic space which is finely granular mottled or striated. Appearing between the tables of the skull the perpendicular striations which seem to extend beyond the outer atrophied table give the appearance of hair standing on end. The earliest skeletal changes are observed in the small bones particularly in the metacarpals and metatarsals.

revealing osteoporosis and expansion of the medullary cavities producing a rectangular rather than a normal concave appearance. Cortical thinning may be so extreme as to result in pathologic fractures.

The character and degree of the bone changes are modified significantly with



Fig. 20. Roentgenograms of a severe case of Cooley's anemia (thalassaemia major). A: Skull showing enlarged diploë space which is fairly granular mottled or striated; note hair-on-end appearance. Marked rarefaction of hand and wrists. B: and of lower extremities. C: D: Note compression of vertebral bodies.

age. In older children the bone lesions regress in the more distal portions of the skeleton (hands, arms and legs) whereas with advancing age red marrow is replaced normally with fatty marrow. The characteristic changes described in the hands and other peripheral areas are thus diminished and may disappear at puberty.¹⁶ On the contrary, in the skull, spine and pelvis which are sites of active and persistent red marrow formation the roentgenographic changes become more conspicuous.^{16,18} Compression of the vertebrae quite common in patients with sickle cell anemia is rare in those with Cooley's anemia. Severe reticulation and rarefaction of the vertebral bodies are not uncommon. Localized tumors of red marrow in the generally hyperplastic marrow have been described. Costal osteomas giving the appearance of large bony masses in the thorax without external signs of tumor have been described.^{19,3} In one of the patients the mass proved to be a large shell of bone containing red marrow and blood.¹⁹ In the patient with the true or mild form of the disease skeletal changes are absent.

Blood Picture The blood picture in patients with thalassemia major and in those with thalassemia minor provides important clues to the diagnosis of these diseases.

Thalassemia Major The anemia is pronounced and of a hypochromic microcytic type similar to that seen in patients with severe iron deficiency. The hemoglobin ranges between 5 and 9 gm per 100 ml and the red cell count between 2.5 and 3.5 million per cubic millimeter. These values are obtained soon after diagnosis before any transfusions have been given and in those patients under treatment after a prolonged interval following transfusion so there is no admixture with donor blood. In patients with the intermediate homozygous type the hemoglobin stabilizes between 7 and 10 gm per 100 ml without the need for transfusions. The reduction in hemoglobin level and hematocrit is proportionally greater than the reduction in the number of red cells as occurs in patient with iron deficiency. The erythrocytes show polychromatophilia, marked hypochromia, anisocytosis and poikilocytosis and are often fragmented.

Although microcytes predominate the most important cells for diagnostic purposes are the large pale erythrocytes interspersed among them which contain irregularly distributed clumps of hemoglobin with the intervening areas apparently possessing staining defects. These cells are extremely thin and leaflike and in wet preparations their edges fold over and the several layers thus formed possess a remarkable transparency. In the stained smear the combination of scattered hemoglobin and the thinness of the cells result in bizarre and wrinkled shapes. The abnormal thinness of the red cells in this disease has led to its designation as hereditary leptocytosis.

A nonspecific macrocytic cell commonly found both in patients with the severe form and in those with mild forms of thalassemia is the target cell. This erythrocyte has been so named because of its deeply stained center and periphery which are arranged in concentric light and dark zones. Another macrocyte is a round or sometimes oval cell with a narrow rim of hemoglobin of varying thickness with a large zone of central achromia in which only a faintly stained island of hemoglobin is occasionally noted.

Nucleated red cells are almost invariably found and constitute one of the most characteristic findings of the disease. The size and maturity of these cells vary but most often they are typical normoblasts and mature micronormoblasts with pyknotic nuclei. Normoblasts vary from a few to large numbers, sometimes equal

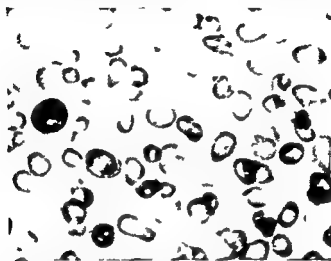


Fig. 21. Photomicrograph of blood smear of patient with severe Cooley's anemia (thalassaemia major) postsplenectomy. Note microcytes, hypochromic macrocytes, anisocytosis, and poikilocytosis. Many of the red cells appear fragmented.



Fig. 22. Blood smear of patient with severe Cooley's anemia, postsplenectomy. Note excessive size and abnormal thinness of red cells, marked hypochromia, and large number of normoblasts.

ing or exceeding the leukocytes numerically Erythroblastosis is extremely marked after splenectomy and constitutes a distinctive postoperative feature

Red cells containing Howell Jolly bodies are infrequently observed in patients with an intact spleen Polychromasia and basophilic stippling are present but are less marked in patients with the severe than in those with the mild type of Cooley's anemia because of the lack of hemoglobin Reticulocytes are increased and range from 2 to 8 per cent¹¹ Moderate leukocytosis is present with white cell counts ranging from 15 000 to 30 000 per cubic millimeter but the upper level may be exceeded Granulocytes predominate and myelocytes are common The platelet count is generally normal Moderate leukopenia and to a lesser extent thrombocytopenia are occasionally observed and attributed to hypersplenisml¹⁰ or suppression by multiple transfusions The bone marrow in severe cases shows primitive erythroid cells and a predominance of basophilic normoblasts microblasts and pronormoblasts

Aplastic crises have been described comparable to those in patients with spherocytic anemia and other hemolytic anemias resulting in erythroid marrow hypoplasia and reticulocytopenia¹²

The osmotic fragility is strikingly abnormal The red cells are markedly resistant to hemolysis in hypotonic sodium chloride solution so that in some instances they are not entirely hemolyzed even in distilled water The thinness of the red cells renders them capable of absorbing more water than normal before disruption The serum bilirubin is usually slightly elevated ranging from 1.5 to 3 mg per 100 ml When the upper limit is exceeded hepatitis cholelithiasis or a hemolytic crisis should be considered The serum iron is increased and the iron binding protein is fully saturated¹³⁴

Thalassemia Minor The essential features consist of hypochromic microcytes and macrocytes basophilic stippling oval and target cells and less frequently polycythemia¹⁰¹ with or without slight anemia Osmotic fragility is greatly decreased even in the absence of anemia

The occurrence of morphologic changes of the red cells far in excess of anemia constitutes a fundamental principle in the detection of thalassemia minor The hemoglobin level in patients with thalassemia minor is above 7 gm per 100 ml Since the mean corpuscular volume of red cells (MCV) represents the ratio between the volume of packed cells and the erythrocyte count the smaller calculated value results from the increased numbers of red cells per cubic millimeter in relation to the normal or slightly lowered volume of packed red cells Hypochromic and microcytic red cells predominate simulating the blood smear of patients with iron deficiency anemia Varying degrees of poikilocytosis and anisocytosis are seen but the distinguishing feature is the interspersal of hypochromic microcytes giving the smear a macrocytic appearance in many cases Target cells appear in fewer than half of the patients oval cells and cylindrical or cigarette shaped forms are common For unexplained reasons patients with the trait or mild anemia frequently show slight eosinophilia

Excessive numbers of erythrocytes with punctate basophilia are sometimes sufficiently pronounced to suggest lead poisoning Occasionally they are equally common in the sibling and one of the parents Elevated red cell counts

exceeding 6 million per cubic millimeter are occasionally noted in children but are most common in adults and are regarded as a compensatory physiologic response. Normoblasts and myelocytes are not present; reticulocytes seldom exceed 5 per cent. We have seen several children with thalassemia minor who had hemoglobin during childhood ranged between 9 and 10 gm per 100 ml and showed a spontaneous rise to 12 and 13 gm during adolescence. Occasionally



Fig. 23 Photomicrograph ($\times 900$) from blood smear of patient with the mild type of Cooley's anemia (thalassemia minor). Note anisocytosis, poikilocytosis, hypochromic macrocytes, microcytes, and oval elongated and rodlike cells. (From Smith C. H. Detection of Mild Types of Mediterranean (Cooley's) Anemia. *Am J D Child* 75:505, 1948.)

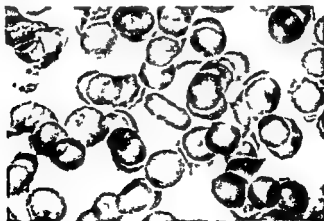


Fig. 24 Photomicrograph ($\times 900$) from patient with mild type of Cooley's anemia. Smear slightly overstained to demonstrate stippling.

this change is accompanied by an elevated red blood cell count. The blood smear however still demonstrated the typical morphologic features of thalassemia. These changes are explained on the basis of the marked elevations of hemoglobin and red cells at times of a polycythemic level which occasionally appear in the normal adolescent.

Erythropoietic hyperplasia of the bone marrow is less marked than in patients with thalassemia major. Polychromatic and pyknotic normoblasts are common.⁷ The serum iron and latent iron binding capacity are normal. Fetal hemoglobin levels are normal.

Hemosiderosis and Hemochromatosis Any disorder which depends upon transfusions as the mainstay of treatment is subject to increased iron deposition. In common with other refractory and chronic hemolytic anemias iron stores accumulate from both the degradation of hemoglobin (250 mg in each 500 ml of blood) and increased iron absorption from the diet by the gastrointestinal tract. Another significant source is from the accelerated destruction of the patient's own defective red cells. In patients with severe Cooley's anemia wide spread visceral hemosiderosis and fibrosis frequently coexist although there is no uniformity about the progression from the former to the latter.⁸ It has been suggested that the development of true hepatic cirrhosis and fibrosis of the pancreas which characterizes hemochromatosis depends upon the intervention of accessory factors such as combined hypoxia in addition to large iron deposits.⁹ Both conditions prevail in patients with severe Cooley's anemia—hence the concern about the development of hemochromatosis. With the advancing age of the child and progressive iron deposition patients will occasionally manifest diabetes and other clinical and pathologic features identical with those of endogenous hemochromatosis. One of the serious complications of Cooley's anemia is irreversible heart failure in which myocardial siderosis plays a major etiologic role.

Red Cell Survival The red cells of the patient with the severe disease transfused into a normal recipient show a half life of about twenty five days as compared with the mean half life of about sixty two days attained with normal erythrocytes.¹⁰ The shortened survival is also noted by the injection of the patient's own red cells labeled with Cr⁵¹ into his own circulation. By this method about 20 per cent of the red cells have a life span of only several days and the remainder of about thirty days. The survival time of erythrocytes from patients with the trait transfused into normal recipients falls within the normal range.¹¹

The use of Cr⁵¹ in determining the longevity of red cells is not impaired by the fact that accelerated rates of Cr⁵¹ elution take place from hemoglobin solutions containing excessive amounts of fetal hemoglobin such as occur in cord blood and in the blood of patients with severe Cooley's anemia. This does not take place however from intact red cells containing large amounts of fetal hemoglobin.¹²

To what extent the neutral mucopolysaccharide found in red cells of patients with severe Cooley's anemia contributes to their shortened life span is unknown.

Diagnosis Thalassemia major possesses so many distinctive features that the diagnosis is rarely overlooked. The association of the characteristic facies, skeletal

changes massive splenomegaly severe anemia markedly hypochromic and distorted red cells stunted growth and usually Mediterranean parentage separates this disease from other hemolytic anemias. Osmotic fragility is decreased and sickling and spherocytosis are absent. The diagnosis is confirmed by examination of the blood of the patient's parents which shows decreased osmotic fragility, basophilic stippling, hypochromic microcytes and macrocytes and oval cells. The child who shows only moderate anemia with many of the clinical features of the severe disease often represents a combination of both sickle cell disease and thalassemia. The marked normoblastemia noted in the blood of patients with severe Cooley's anemia has led to confusion with de Guglielmo's disease, a rare entity in childhood. The occurrence of this erythroid proliferative disorder in a child with thalassemia has been described.¹³⁴

Thalassemia minor resembles iron deficiency so closely that the two are usually indistinguishable. The blood smears are identical and show large numbers of hypochromic microcytes, moderate stippling and oval and target forms. The diagnosis is made by elimination since there are no pathognomonic features as are observed in patients with thalassemia major. Frequently a diagnosis of thalassemia minor is suggested when a patient believed to have iron deficiency anemia has not responded to iron therapy. The discovery in at least one parent of a blood picture similar to that of the patient, an abnormal percentage of A hemoglobin and a normal serum iron content as contrasted with a low serum iron content in patients with iron-deficiency anemia establish the diagnosis of thalassemia minor. In the adult patient thalassemia minor may coexist with pernicious anemia³⁴ or megaloblastic states.⁶

Starch Block Electrophoresis of Hemoglobin in the Diagnosis of the Thalassemia Trait When hemoglobin analyses are performed by paper electrophoresis at a pH of 8.6, two subtypes of normal hemoglobin are found. In addition to the main component A₁, slow and fast components appear which are designated as A and A₂, respectively. By the Tiselius moving boundary method performed at a pH of 6.5, A₁ in contrast to its reaction during paper electrophoresis migrates faster than A₁.¹⁶⁶

An alternate method in which a starch block prepared from potato starch powder is substituted and hemolysates prepared in an identical manner as in the paper method are used provides electrophoretic patterns of diagnostic value in patients with thalassemia.^{64, 109, 166}

The starch block suspended in an arbitrarily set volume of barbital buffer at a pH of 8.6 (ionic strength 0.05) is covered with Phofilm and transferred to a cold room where electrophoresis is carried out at 4°C at a specified voltage and current flow. Three major hemoglobin fractions can be readily observed, A₁, A₂, and A, in order of their speed of migration toward the anode. The A component resembles hemoglobin E in electrophoretic mobility. The percentage of each component of A is obtained by elution from the respective areas of the block and determined spectrophotometrically. A is found in a concentration of approximately 1 to 3 per cent in the blood of normal persons. Levels in excess of 3.1 per cent have been shown to have diagnostic value for the thalassemia trait.^{133, 176}

this change is accompanied by an elevated red blood cell count. The blood smear, however, still demonstrated the typical morphologic features of thalassemia. These changes are explained on the basis of the marked elevations of hemoglobin and red cells at times of a polycythemic level which occasionally appear in the normal adolescent.

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Diagnosis. Thalassemia major possesses so many distinctive features that the diagnosis is rarely overlooked. The association of the characteristic facies, skeletal

years ago because of the sudden development of a hemolytic component after a burn necessitating periodic transfusions. Since splenectomy the hemoglobin concentration is stabilized at 9 to 10 gm per 100 ml without transfusions although the blood smear still shows marked normoblastemia and other typical red cell changes characteristic of severe Cooley's anemia.

The frequently made statement that thalassemia major is almost always fatal before puberty no longer holds within the accepted definition of this term. It is difficult however to designate the precise period of puberty in these patients since the development of sexual maturity is greatly retarded. Whereas infection was responsible for the death of many children with severe Cooley's anemia in the years before antibiotics were available they now succumb to heart failure. Although long term transfusions prolong the lives of these patients they are nevertheless responsible in large part for increased tissue iron. That excessive absorption of iron is also important as a cause of hemosiderosis is emphasized in those patients with thalassemia who have received minimal transfusions and eventually succumbed to heart disease.¹ In large part cardiac complications are related to the heavy deposition of iron in the myocardium and are comparable in this respect to a similar incidence in idiopathic hemochromatosis.⁶¹ Once heart failure sets in it is irreversible and the maximal duration of life from the onset of symptoms is approximately one year. The initial sign of a threatening cardiac nature in the asymptomatic patient is prolonged auricular-ventricular conduction (P-R interval) and the appearance of atrial premature contractions. With manifest heart failure more serious atrial as well as ventricular arrhythmias occurred.

Acute benign nonspecific pericarditis has been described in thalassemia especially among patients who have been splenectomized.¹⁷ In all patients the pericarditis was self limited and ended in complete recovery. The basis for this peculiar susceptibility is unknown.

Treatment There is no specific treatment for patients with severe Cooley's anemia. Iron, copper, liver extracts from the spleen, pancreas and adrenal gland and other endocrine products, steroids, large dosages of vitamin B components, plasma and cell extracts and x-ray therapy have all been used without effect. Since only questionable improvement has been reported with cobalt¹⁸ it is not employed in routine treatment. Cooley's anemia represents a hypochromic microcytic anemia in which iron therapy is without value as would be expected from the abundant iron stores already present.

Transfusions Transfusions are mainly relied upon as treatment for patients with thalassemia and when they are excessive splenectomy is necessary. Hemoglobin and red blood cell levels in children with severe Cooley's anemia continue to decline progressively so that supportive transfusions are required to keep the patients asymptomatic under the stress of normal activity. Usually this necessitates the administration of blood at two- to three week intervals to maintain minimal levels. Transfusions however required to support adequate hemoglobin levels carry the inherent drawback of increased iron deposition from degraded hemoglobin. The hemoglobin level at which transfusions are indicated cannot be arbitrarily fixed but varies with the associated signs and symptoms in the individual patient. As a guide to management patients with severe

A is increased in patients with thalassemia minor with a range of 2 to 9 per cent and a mean of 5.11 ± 1.36 which exceeds the mean value in patients with thalassemia major and other miscellaneous diseases.¹⁰ The elevation of the A component serves therefore to distinguish patients with thalassemia minor from normal persons. Maximal values have occasionally been found in patients with intermediate forms of thalassemia and rarely in those with the severe disease. It should be emphasized that although the majority of patients with thalassemia minor show elevated A concentrations a small percentage possess a normal concentration of this component. In this connection it has been pointed out¹¹ that the thalassemia gene that is not associated with an elevated A fraction may also be responsible for the failure to interact with the gene for sickling hemoglobin C and hemoglobin E. Ordinarily the simultaneous presence of the thalassemia gene (presumably elevated A) in combination with the gene for sickling results in a severe anemia resembling homozygous rather than heterozygous sickle cell anemia. Two types of thalassemia have therefore been postulated based upon high and low A.

Although starch block electrophoresis provides definitive separation and measurement of A, hemoglobin paper electrophoresis by far the simpler method serves a similar function especially where this pigment is present in substantial amounts. In our laboratory the normal A₂ range with the paper method was 5 to 14.3 per cent as compared with 13.2 to 27 per cent in patients known to have heterozygous thalassemia.¹² Only these values for A which lie above the limit of overlap in both paper and starch methods are diagnostic for the heterozygous thalassemia state.

Course and Prognosis Patients with thalassemia minor live normally whether or not mild anemia is present. The outlook for patients with thalassemia major is poor but the course is not a uniform one and depends upon the grade of severity of the disease encountered in the individual patient. As has been stated previously thalassemia major falls into two classifications, the severe type in which the patients require transfusions to maintain hemoglobin levels commensurate with normal activity and a less severe one, the so called intermediate type in which patients function effectively without transfusions maintaining hemoglobin levels of approximately 7 to 9 gm per 100 ml. This separation can usually be made clinically but transitional cases occur in which hemoglobin concentrations of patients requiring frequent transfusions subsequently stabilize at adequate levels without further treatment. On the other hand patients with intermediate disease are observed who require no transfusions until there is exposure to infection or other noxious agents and more frequently for no apparent reason. From this point on transfusions may be required so frequently that splenectomy is considered necessary.

Multiple transfusions at carefully spaced intervals, splenectomy in selected patients and the liberal use of antibiotics permit a better prognosis for longevity in patients with thalassemia major than had heretofore been considered. Since the introduction of these combined measures is relatively recent their effect on ultimate longevity still has to be determined especially in patients with the intermediate type of disease. In either classification it is rare for severely affected patients to survive beyond the third decade. Among the patients with thalassemia major in our series the oldest is now 24 years old and among those with the intermediate type of disease there are three at least who are in their thirties. One of these now 37 years old originally reported on when he was an adolescent¹³ has three children. This patient's spleen was removed three

whelming infection following splenectomy in patients with Cooley's anemia and other dyscrasias in childhood.^{66, 91, 105, 115} In comparison with the necessary numbers of splenectomies the incidence is avowedly small. Nevertheless close supervision for several years postoperatively is essential so that appropriate treatment may be instituted immediately in the event of an infection.

There is no contraindication to tonsillectomy in patients with Cooley's anemia. Minor coagulation defects may coexist with structural and functional abnormalities of the liver.

Homozygous Hemoglobin C Disease

Homozygous hemoglobin C disease is a rare condition occurring principally in Negroes and occasionally in Caucasians.

Essential Features. Anemia is absent or mildly hemolytic; the reticulocyte count is normal or slightly increased and bilirubin may be slightly elevated—features indicative of a compensated hemolytic process. The red cells are normocytic or slightly microcytic and normochromic; normoblasts appear occasionally and tetragonal crystals of hemoglobin have been observed in 2 per cent of the erythrocytes in one patient.⁴¹ Red cells show a decreased osmotic fragility. Moderate erythrocytic hyperplasia is present in the bone marrow. The striking feature of the blood film is the large number of target cells estimated at 40 to 100 per cent of all erythrocytes. Paper electrophoresis reveals 100 per cent hemoglobin C which has the slowest mobility rate of all hemoglobins tested by this technique. With rare exceptions fetal hemoglobin is not elevated above normal levels. As in patients with other hereditary hemolytic syndromes the red cells of patients with homozygous hemoglobin C disease injected into a normal recipient show a shortened survival. The exponential type of survival time curve indicates the random destruction of red cells regardless of their age.¹⁶⁸ Splenomegaly is either moderate or very marked. Abdominal pain, arthralgia, jaundice and cholelithiasis are uncommon. The prognosis is good, and transfusions are rarely necessary. Splenectomy is occasionally carried out for relief of symptoms relating to pain over the splenic area.⁴⁵

Hemoglobin C Trait

The combination of hemoglobins A and C is found in the asymptomatic carrier who is identified by the reaction of his blood to electrophoresis and the presence of numerous target cells in the blood smear. In the person with the trait the C pigment varies from 28 to 44 per cent; sickling is absent; fetal hemoglobin is normal and a mild hypochromia may be present. The hemoglobin C trait is detected in about 2 per cent of the American Negro population.

Hereditary Elliptocytosis and Hemoglobin C Trait. Two children in a Negro family were found to have both elliptocytosis and hemoglobin C. Each of these factors was traced to the respective parents. In one child some of the target cells associated with the hemoglobin C trait were elliptical, indicating the combined genetic effects in the same cells. Anemia in one child was corrected by iron and the red cell survival was normal. The combination of elliptocytosis and hemoglobin C trait does not show a summation effect.⁴

Cooley's anemia do not require transfusions until hemoglobin levels drop between 6.5 and 7.5 gm per 100 ml at which point clinical symptoms frequently set in.

In our experience maintaining hemoglobin levels below 6 gm per 100 ml is potentially hazardous from the standpoint of developing heart failure. In patients with established cardiac difficulties the hemoglobin level should be maintained between 8 and 9 gm per 100 ml to prevent hypoxia. In addition to transfusion hemosiderosis another deterrent to excessive blood administration is the possibility that multiple transfusions may depress endogenous hemoglobin synthesis and red cell function.¹⁻³

Until specific treatment becomes available the most serious problem encountered in the patient with severe Cooley's anemia is the prevention and control of congestive heart failure. Treatment consists of the usual means of combating heart failure and controlling arrhythmias—namely digitalization, diuretics, low salt diet, quinidine, etc. In addition small transfusions of packed red cells are given. At present there is no effective measure to rid the heart of excessive iron. Mobilization of iron stores achieved by repeated phlebotomies in patients with idiopathic hemochromatosis is precluded in patients with Cooley's anemia because of the constant need for maintaining adequate hemoglobin levels. Chelating agents which increase urinary excretion of iron are important in the treatment of patients with conditions such as severe Cooley's anemia in whom massive iron loads exist.^{6,7}

Splenectomy. In the severely affected person the need for splenectomy is clearly indicated when transfusion requirements are progressively increased and when the spleen becomes massive and burdensome causing discomfort and symptoms due to pressure. Although efforts are made in the immediate pre-splenectomy period to vary the number and size of transfusions so as to defer splenectomy, the blood requirements nevertheless become so extreme that decision for the operation is usually forced.¹

At this time there will usually be abundant evidence that a hemolytic factor has developed which shortens the life of normal transfused cells. When there is doubt labeling donor cells with radioactive chromium will establish the rate at which transfused blood is being destroyed. Although the increasing need for transfusions before splenectomy and the sharp reduction afterward confirms the elevation of an extracorporeal hemolytic component it does not appear to alter the basic disease significantly.^{1,7} Retardation of growth and the incidence of congestive heart failure are not altered by removal of the spleen. From available data splenectomy appears more effective in the older child but this may reflect a genetically milder disease permitting postponement until puberty. Another explanation is that slowing of growth and diminished metabolic needs in adolescence permit the establishment of erythropoietic equilibrium at levels compatible with well being without supplementation by transfusions. Despite the preference to delay splenectomy until adolescence it is frequently necessary to remove the spleen of the younger patient because of excessive transfusion needs.

Recent experience has demonstrated a potential hazard of severe and over

Hemoglobin G

Hemoglobin G pigment moves more slowly than hemoglobin A and faster than hemoglobin S corresponding electrophoretically to the area in which fetal hemoglobin is found.^{4, 4} Its mobility coincides with fetal hemoglobin or migrates somewhat more slowly. Hemoglobin G is not alkali resistant and can be differentiated from fetal hemoglobin in this manner.^{1, 2}

Hemoglobin G Trait In a family from the African Gold Coast in which the parents were homozygous for hemoglobins A and G respectively, nine siblings were heterozygous (A G) as expected.^{4, 4} These persons with the trait presented no clinical or hematologic abnormalities.

Homozygous Hemoglobin G Disease The father of the foregoing family (GG) was asymptomatic and did not have anemia (hemoglobin 13.9 gm) and the blood smear was free of abnormalities. Fetal hemoglobin was not present, reticulocytes were 2 per cent and the serum bilirubin level was normal.^{4, 4}

Hemoglobin G-Sickle Cell Disease With heterozygous hemoglobin G sickle cell disease a moderately severe hemolytic anemia occurs with splenomegaly, sickling, increased osmotic resistance to hypotonic saline solution and a blood picture resembling sickle cell thalassemia disease.^{1, 2}

Hemoglobin G-Thalassemia An Italian patient with hemoglobin G-thalassemia possessed a blood picture and clinical findings somewhat more severe than those of patients with thalassemia minor. The hemoglobin was 10 gm and the smear revealed hypochromic red cells, target cells and basophilic stippling. Other features included no elevation in fetal hemoglobin, slight reticulocytosis, a palpable spleen and moderately hyperplastic bone marrow. The red cell survival was about two thirds of the normal.¹ In an American family of Italian origin hemoglobin G, hemoglobin S and thalassemia occurred together.^{1, 2} Hemoglobin G did not appear clinically important taken alone or in combination with hemoglobin S or thalassemia.

Hemoglobin H

Three members of a Chinese family were found to have a hypochromic microcytic anemia with blood smears resembling thalassemia. The electrophoretic pattern revealed an abnormal hemoglobin migrating more rapidly than normal hemoglobin (A). Other features included refractoriness to iron therapy, reticulocytosis, poikilocytosis, intraerythrocytic inclusion bodies, decreased osmotic fragility, a shortened red cell survival and splenomegaly.^{9, 11} The abnormal hemoglobin accounted for 35 to 40 per cent of the total; fetal hemoglobin was slightly increased and the remainder was normal hemoglobin. It is of interest that this fast moving hemoglobin did not appear in either parent and that the father and daughter of one of the affected patients showed red cell changes consistent with thalassemia minor.¹¹ In all subsequent reports the same failure to find hemoglobin H in either parent was observed.

A similar blood picture interpreted as hemoglobin H-thalassemia disease has been described in two unrelated middle aged Filipino men. Hemoglobin analysis in these cases also revealed a major component of normal hemoglobin and a minor component of hemoglobin H. Fetal hemoglobin was normal.¹²

Hemoglobin D

Hemoglobin D is a rare hemoglobin which possesses an electrophoretic mobility identical with sickle hemoglobin. It is distinguished by its much higher solubility in reduced form than the latter in a comparable physical state. In contrast to homozygous hemoglobin S disease gelling, trictoid formation and sickling are absent. Homozygous hemoglobin D disease has rarely been reported.¹⁻⁴ The anemia is slight, target cells are numerous, osmotic resistance to hypotonic saline solution is increased, and the erythrocyte life span is shortened.

The person with the trait (hemoglobins A and D) is asymptomatic and reveals no quantitative blood changes and no sickling. He can only be identified by the presence of hemoglobin D by electrophoresis. Hemoglobin D is not uncommon among American Negroes with a reported incidence of 0.4 per cent.¹ Foci of hemoglobin D prevalence have been described in Algerian Moslems and Indians of North Central India.^{4a}

Hemoglobin E Disease

Hemoglobin E disease was discovered during an investigation of a group of severely anemic patients from Thailand whose clinical and hematologic characteristics closely resembled those of patients with severe thalassemia. In some of these patients a mild course and survival into adult life suggested deviation from the uniformly severe case of thalassemia. A significant feature was the presence of the trait of thalassemia in only one parent instead of in both, which was to be expected in the usual case of the severe disease in a child. Investigation revealed that these patients possessed an additional hemoglobin designated as compound E which was also present in the second parent. With paper electrophoresis it was possible to demonstrate that hemoglobin E had a mobility intermediate between hemoglobin C and hemoglobin S. Further studies¹³⁰ revealed the several categories of pure or homozygous hemoglobin E disease, hemoglobin E trait (hemoglobin A plus hemoglobin E), and thalassemia-hemoglobin E disease. A survey in connection with these studies revealed that hemoglobin E was found in 13 per cent of persons of Thai extraction but was not detected in any of the racially unmixed Chinese in that area.

Homozygous Hemoglobin E Disease. The clinical features include easy fatigability, mild jaundice, and occasionally icterus. In the small group of cases in this category the spleen was slightly enlarged in only one patient and the liver in another. The hemoglobin level is moderately lowered, the erythrocyte levels are normal, reticulocytes are not increased, and target cells are numerous (25 to 60 per cent). Red cell constants indicate a microcytic hypochromic anemia. The red cells show an increased resistance to hemolysis in hypotonic saline solutions. The bone marrow shows a mild erythrocytic hyperplasia. Paper electrophoresis reveals 94 to 95 per cent hemoglobin E and up to 5 per cent fetal hemoglobin.

Hemoglobin E Trait. Persons with the trait are asymptomatic. Blood values are normal and target cells are not observed. Paper electrophoresis reveals two components, normal hemoglobin A and hemoglobin E. Normal amounts of fetal hemoglobin are demonstrated by alkali denaturation.

normal hemoglobin Sicking was absent and no hematologic or physical abnormalities were found.¹³⁹

Two hemoglobin types have been observed in Liberian natives—one with a mobility corresponding to hemoglobin J and the other between hemoglobin J and A. No cytologic abnormalities were found in either patient.¹⁴⁰

Hemoglobin M

The designation of hemoglobin M was proposed¹⁴⁶ for a spectroscopically abnormal methemoglobin first described by Horlein and Weber⁴⁸ in a family with hereditary methemoglobinemia. They showed that the cyanosis was due to the presence of methemoglobin with an unusual spectral characteristic which was attributable to abnormality of the globin. Subsequently, Gerald⁶⁷ found that the hemoglobin from a patient with hemoglobin M disease could be resolved into two components identified electrophoretically and spectroscopically—one is hemoglobin A and the other is hemoglobin M. The nature of the spectral changes indicates that some of the heme groups of the methemoglobin form react abnormally and others apparently normally.

In contrast to the therapeutic effect of methylene blue and ascorbic acid in patients with the established forms of congenital and of drug-induced types of methemoglobinemia in converting the pigment back to normal hemoglobin, methemoglobinemia due to hemoglobin M is unaffected by this therapy. Patients with the abnormal hemoglobin M have approximately 15 to 20 per cent of the total pigment in the form of methemoglobin and are seemingly unaffected by its presence.¹⁴⁷

Miscellaneous Abnormal Hemoglobins

Several additional hemoglobinopathies reported from isolated areas thus far have only local significance. They are characterized by the following mobilities on the basis of paper electrophoresis at pH 8.6. Hemoglobin K moves faster than hemoglobin A and is so closely attached to the latter that it appears as its prolongation.¹⁴⁸ The abnormal component moves slower than hemoglobin J. Hemoglobin L¹ migrates between hemoglobin S and hemoglobin G. Hemoglobin N¹⁴ moves faster than hemoglobin J but more slowly than hemoglobin H. Hemoglobin O migrates between hemoglobin S and hemoglobin E. Hemoglobin Q^{19,20} migrates in largest part between hemoglobin A and hemoglobin S, the remainder consisting of hemoglobin H. In contrast to patients with other hemoglobins listed in the miscellaneous category, the patient with hemoglobin Q disease suffered with a hypochromic anemia refractory to treatment.

SICKLE CELL VARIANTS

Sickle Cell—Thalassemia Disease (Microcytic Anemia)

Sickle cell-thalassemia disease (microcytic anemia) accounts for most of the cases of sickle cell disease in white persons (usually of Greek or Italian origin) although it has been reported in American Negroes.¹⁴⁹ Such a patient usually inherits the gene for thalassemia from one parent and the gene for sickle cell hemoglobin from the other. Rare exceptions¹⁵⁰ include instances

Increasing numbers of patients with hemoglobin H in combination with thalassemia minor (hemoglobin H-thalassemia) have been observed.^{11, 16, 64} Cases of hemoglobin H have been found not only in Chinese and Filipinos but also in Thai, Greek, Italian, and Swedish patients. Although devoid of hemoglobin H, parents and siblings in these reports present evidence of the thalassemia trait as well as of intrerythrocytic inclusion bodies resembling Heinz bodies. Further testing for increases of A hemoglobin is necessary to confirm the diagnosis of thalassemia minor in these patients.²

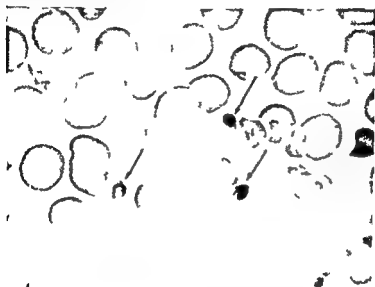


Fig. 25 Blood smear from a patient with hemoglobin H disease. Note inclusion bodies in three erythrocytes. (Courtesy Dr Ralph L. Engle, Jr., New York, N. Y.)

Chronic hypochromic microcytic anemia associated with the presence of intrerythrocytic crystals and hemoglobin H has been described. The parents did not show the abnormal hemoglobin H in their blood. The family was partly of Chinese and partly of Indonesian origin.

Hemoglobin I

A hemoglobin with a mobility more rapid than normal hemoglobin A was detected in several members of a Negro family. The affected persons were asymptomatic and showed no hematologic abnormalities.¹⁴ This hemoglobin was present in equal amounts with hemoglobin A. Differences in mobility between hemoglobin H and hemoglobin I were not apparent at a pH of 8.6 but were clearly distinguishable at a pH of 6.5.

Hemoglobin J

Hemoglobin J, observed in a Negro family, was shown to have a more rapid electrophoretic mobility at a pH of 8.6 than normal adult hemoglobin but a slower mobility than the next component I. It was found in combination with

is a moderate form of Cooley's anemia rarely of sickle cell anemia. Anemia is moderate with hemoglobin levels of 6 to 9 gm per 100 ml usually about 8 gm and red cells number approximately 3 million per cubic millimeter. Red cell constants indicate a microcytic hypochromic anemia as occurs in patients with thalassemia. The other sickle cell variants show normochromic normocytic red cell constants as in homozygous (SS) sickle cell anemia.

Clinical Findings. The clinical manifestations are less severe than in patients with either sickle cell anemia or thalassemia major. Crises are rare but abdominal bone and joint pain and at times unexplained fever occur. Severe cases are occasionally observed as in patients with sickle cell anemia. Jaundice is mild and splenomegaly is mild to moderate. Aseptic necrosis of the head of the femur has also been reported¹¹. Splenomegaly is much more common than in patients with sickle cell anemia but not so marked as in those with severe Cooley's anemia. Hepatomegaly may be of significant degree. Patients with this disease are recognized because of anemia and later in childhood splenomegaly unlike those with thalassemia major in whom these features are already advanced in the first year of life. Treatment depends upon severity. In severe crises multiple transfusions are needed and splenectomy rarely if blood requirements are high. Patients with mild cases require no treatment. Prognosis is better than in patients with homozygous sickle cell anemia.

Sickle Cell-Hemoglobin C Disease

The simultaneous presence of the sickle cell gene and the gene for hemoglobin C results in a type of sickle cell disease second in frequency only to classical sickle cell anemia among American Negroes.¹⁻⁴ The evidence derived from genetic studies strongly points to the association of these genes as alleles or close linkage upon the same chromosomes.¹¹⁹

The clinical manifestations are usually of lesser severity than those in patients with homozygous sickle cell anemia but are extremely variable ranging from an asymptomatic state to severe disability. Some of those patients heretofore regarded as having milder cases of sickle cell anemia probably belong in the category of hemoglobin S C disease. Crises are of lesser severity and are infrequent. Hepatomegaly is usually present and splenomegaly is moderate or marked. Fatigue, dyspnea, jaundice, migratory arthralgia and recurrent abdominal pain may be present. Pregnancy seems to aggravate the clinical and hematologic signs and carries a greater risk than classical sickle cell (SS) anemia. Hematuria, aseptic necrosis of the head of the femur and splenic infarction during aerial flights have been described in hemoglobin S C disease.¹⁸⁰ The course varies markedly in different patients and in individual patients.^{19, 20}

The anemia is usually of a mild normochromic type and the red cells vary slightly in size being either normal or decreased in volume. The hemoglobin level rarely falls below 8 gm usually ranging from 9 to 10 gm and red cells range between 3.5 and 4.5 million per cubic millimeter. Anisocytosis and poikilocytosis are occasionally noted in patients with severe disease. Crises are associated with normoblastosis, reticulocytosis and increased numbers of white cells in the peripheral blood. The reticulocyte count generally is normal or slightly elevated.

in which one parent with sickle cell-thalassemia disease (the other parent being normal) transmits both of the abnormal factors to the offspring. In another instance a normal offspring resulted from one parent with the combined disease and the other normal. Electrophoretic studies reveal the presence of sickle, normal and occasionally fetal hemoglobin. The percentage of sickle hemoglobin ranges from 65 to 80 per cent (in one patient 90 per cent) which is less than the 75 to more than 85 per cent present in homozygous sickle cell anemia.¹ Hemoglobin analyses by electrophoresis and alkali denaturation have demonstrated that thalassemia-sickle cell disease consists of hemoglobins S, A and F or hemoglobins S and A. Homozygous sickle cell anemia on the other hand consists exclusively of sickle and fetal hemoglobins, the latter in amounts of 2 to 24 per cent.¹⁶ The higher percentage of sickle cell hemoglobin in the offspring than in the parent with the trait (25 to 45 per cent hemoglobin S) reflects the potentiating effect of the associated thalassemia gene. The disproportionate amount of hemoglobin S despite its presence in only one parent may also stem from the depression of normal hemoglobin synthesis by the thalassemia gene with corresponding increase of the abnormal hemoglobin. The clinical manifestations of the combined disease represent predominantly a sickle type of hemolytic anemia although of lesser severity than the homozygous sickle cell disease.



Fig. 26 Blood smear from a patient with sickle cell thalassemia disease ($\times 700$). Note hypochromic microcytes and tendency for oval cell, target cells and sickle cell to occur.

Essential Features The blood smear shows thin hypochromic microcytes, poikilocytosis, marked anisocytosis, oval cells, basophilic stippling and less frequently normoblasts. The increase in target cells exceeds that in patients with sickle cell anemia. Sickle cells are few in number in the fixed smear but are increased at times to 100 per cent in sickle cell preparations. Osmotic fragility is decreased. Clinically and hematologically the disease presents itself

hemoglobin has been found. The liver and spleen may not be palpable or both organs may be greatly enlarged. Sick cell hemoglobin D disease has been reported in an 18 year old Negro male.¹¹¹

THALASSEMIA VARIANTS

Thalassemia-Hemoglobin C Disease

Thalassemia-hemoglobin C disease usually occurs in Negroes and exceptionally in white persons. The anemia varies in intensity; it may be mild, rarely severe—in which case a hemoglobin level of 7 to 9.5 gm is observed. In the latter instance it runs the course of a severe chronic hypochromic microcytic anemia although milder than that in patients with the homozygous thalassemia disease. Usually

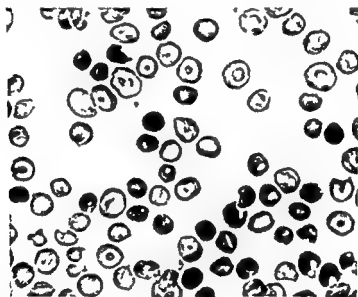


Fig. 27. Blood smear from patients with thalassemia-hemoglobin C disease. Note large number of target cells and microspherocytes. (From Erdanson, M., Smith, C. H., and Schulman, I.: *Thalassemia-Hemoglobin C Disease*. White Siblings, Pediatrics 17:740, 1958.)

however, there is no anemia and reticulocyte count is not elevated. Osmotic fragility may be normal, but in a few cases increased resistance to hypotonic saline solution has been noted. The blood smear shows many target cells (20 to 60 per cent), anisocytosis and poikilocytosis, and in appreciable number of microspherocytes.¹¹² Since the percentage of target cells in patients with severe Cooley's anemia is usually less than 10 per cent, the finding of an unusually large percentage of these cells should lead one to suspect a hemoglobin variant of this disease. Reticulocytes number 2 to 5 per cent. Fetal hemoglobin is not elevated. Hepatosplenomegaly is either absent or moderate. The disease results from the interaction of the genes for sickling and thalassemia inherited from respective parents bearing the trait. In this combination also hemoglobin C usually is present in

serum bilirubin is normal and osmotic fragility is decreased. Erythroid hyperplasia which characterizes the bone marrow is not so marked as in the bone marrow of patients with homozygous sickle cell anemia.¹⁰⁷ The absence of anemia in many children reflects compensatory hyperactivity of the bone marrow and is indicated by an increased reticulocyte level. Sick cells are few or absent in the blood smear but rapid and complete sickling occurs in sealed fresh preparations. Target cells are numerous averaging 60 per cent (40 to 85 per cent). This feature is of some significance since excessive numbers of target cells in the blood smear of a patient with a mild form of sickle cell anemia should arouse suspicion of the coexistence of hemoglobin C.

In this disorder in which the patient is heterozygous for both abnormal hemoglobins each of the components may be regarded as equally divided,¹¹⁸ although hemoglobin C may range from 37 to 67 per cent. Fetal hemoglobin has been found in amounts up to 7 per cent. Transfusions may be required in episodes of severe hemolytic anemia and splenectomy has been employed in some of the patients in whom splenic infarction developed during flight.¹⁶¹

Sickle Cell—Hereditary Spherocytosis

Sickle anemia in combination with congenital spherocytosis has been observed with improvement of the anemia following splenectomy.¹¹ Sickling remained unaffected with a residual blood pattern showing the sickle cell trait (hemoglobin A S). Each of the constitutional red cell defects (spherocytes and sickling) could be traced to individual patients.^{98, 119, 120}

Hereditary Spherocytosis, Sickling and Thalassemia. An American Negro family has been described⁸ in whom was found the genes responsible for three inherited abnormalities of the erythrocytes: spherocytosis, a thalassemia like trait and the sickling phenomenon. One patient was the possessor of the three abnormal genes. Two (spherocytosis and sickling) were observed in blood examination whereas the presence of the third (thalassemia) was inferred from its occurrence in two of the patient's children. The A hemoglobin in this family was not increased. Analysis of the family pedigree suggests an independent and nonallelic segregation of the genes for each condition.

Sickle Cell—Hemoglobin D Disease

The patients with sickle cell hemoglobin D disease are white persons in whom the coexistence of hemoglobins S and D results in a moderately severe hemolytic process similar to that observed in patients with sickle cell anemia.¹²¹ However one parent reveals the sickle cell trait and the other the trait of hemoglobin D. The blood count reveals a moderate anemia with a hemoglobin level of about 8 to 9 gm per 100 ml of blood and a red cell count of approximately 2.5 million per cubic millimeter. The peripheral blood may show poikilocytosis, anisocytosis, polychromasia and occasionally nucleated red cells. In two reported patients¹²² the red cells were increased in size. A few partially sickled cells are found in the fixed smear. With sickling tests the holly leaf or slow variety of sickling occurs rather than the filamentous or rapid variety found in patients with classical sickle cell anemia. Target cells are infrequent. Up to 12 per cent fetal

cytic hyperplasia. Hemoglobin analysis shows a mixture of hemoglobins E and F with the former comprising 60 to 80 per cent of the total and fetal hemoglobin the remainder. When complete studies have been available one parent possessed hemoglobin E and the other showed hematologic evidences of the thalassemia trait.

Transfusions are necessary in many of the younger children but for the entire group they are not so urgent as in patients with severe Cooley's anemia. Splenectomy in many of these patients has been of benefit in promoting growth and better general health.

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Variants of thalassemia with hemoglobins G and H are described elsewhere in this book.

### **Thalassemia—Lepore Hemoglobin**

In the patient with the Lepore trait the blood picture resembles that of the patient with the trait of thalassemia (slight hypochromia microcytosis and leptocytosis) except for a low content of A hemoglobin. The abnormal (Lepore) hemoglobin is detected by starch electrophoresis and not by paper electrophoresis. At a pH of 8.6 it migrates in the region of sickle hemoglobin. It represents 10 to 12 per cent of the total pigment. In the cases reported<sup>4</sup> patients inherited the Lepore and the thalassemia traits from the respective parents. The Lepore trait-thalassemia trait interaction results in a moderately severe hemolytic anemia with marrow hypertrophy secondary bone changes and moderate splenomegaly but not so extreme as in patients with thalassemia major. The hemoglobin level ranges between 6 and 10 gm per 100 ml and the erythrocyte morphology resembles that of thalassemia consisting of marked microcytosis hypochromia and many target cells.<sup>1, 6</sup>

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an amount greatly in excess of that found in the parent and reflects the effect of coexistence with the thalassemia gene.<sup>163</sup> In the reported patients<sup>163</sup> of hemoglobin C ranged from 29 to 93 per cent

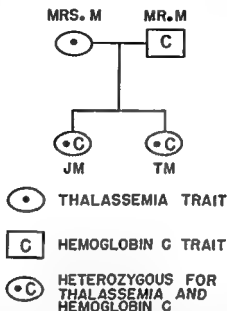


Fig. 28 Example of a family pedigree showing genetic transmission of double heterozygosity for two different sets of gene—thalassemia and hemoglobin C (From Smith C H. The Abnormal Hemoglobins: Clinical and Hematologic Aspects. J Pediat 50:91, 1957, based on data from Erlandson M, Smith C H, and Schulman I. Thalassemia-Hemoglobin C Disease in White Siblings. Pediatrics 17:740, 1956.)

### *Thalassemia—Hemoglobin E Disease*<sup>156</sup>

Thalassemia-hemoglobin E disease represents a severe hemolytic syndrome simulating advanced Cooley's anemia (thalassemia major) so closely as to be indistinguishable from it except that the course may be somewhat milder. Most cases of the combined disease have their onset in infancy, although medical attention may not be sought until adolescence. Fatigue, dyspnea on effort, joint pains, pallor, and jaundice occur frequently. The abdomen is markedly protruberant, the extremities are wasted, and jaundice varies in severity. Splenomegaly may be massive and hepatomegaly is less marked. The patients are of small stature and present a facial appearance with mongoloid features similar to those of patients with classical Cooley's anemia.

Hematologic examination reveals a microcytic hypochromic hemolytic anemia with markedly lowered hemoglobin and red cell values. The reticulocyte count is elevated. The blood smear coincides with that of the patients with the correspondingly severe Cooley's anemia. In some patients the stained blood smear reveals target cells numbering 5 to 20 per cent of the red cells and a small number of spherocytes. Although osmotic fragility starts in more concentrated solutions of sodium chloride than is normal, fragility is not complete until the lowest dilutions are reached. Nucleated red cells are prominent in the blood smear. The bone marrow, like that in patients with Cooley's anemia, shows marked erythro-

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## *Polycythemia, Methemoglobinemia, Sulfhemoglobinemia, and Miscellaneous Anemias*

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### POLYCYTHEMIA

Polycythemia refers to an increase in the number of red blood cells in the hemoglobin level and in the hematocrit per unit volume of blood which is substantially in excess of normal values. Several types of polycythemia may be differentiated: *true or absolute polycythemia* in which the total number of red cells (red cell mass) in the circulating blood is increased; and *relative polycythemia* resulting from the shrinkage of the total plasma volume in which the red cell volume remains normal.

True polycythemia may be further classified into *primary polycythemia* (polycythemia vera) of unknown etiology and *secondary polycythemia* in which there is an increase in total volume of red cells in response to a recognizable cause. The latter includes conditions in which oxygen saturation of arterial blood is lowered, pathologic states involving the central nervous system and abnormalities of pigment metabolism. Except in persons with polycythemia due to hemoconcentration, the blood volume is increased chiefly as a result of augmentation of circulating red cells.

The alkaline phosphatase activity in the polymorphonuclear cell serves as a differential test between the two types of polycythemia. A strongly positive test is observed in persons with leukemoid reactions due to polycythemia vera and a negative test signifies myelogenous leukemia. Secondary polycythemia unassociated with a leukemoid response falls within the range of normal.<sup>11,12</sup>

### *Relative Polycythemia*

Relative polycythemia is observed in persons with conditions of hemoconcentration associated with marked loss of body fluids as occurs in patients with diarrhea, vomiting, severe burns, profuse sweating, and dehydration from water deprivation. The "polycythemia of stress" occurring in anxiety states in adults is also due to reduced blood volume.<sup>1,2</sup> In patients with relative polycythemia the white blood cells and platelets are normal.

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associated with cyanotic congenital heart disease there is no persistence of fetal hemoglobin and in contrast to polycythemia vera the leukocyte and platelet counts are not increased. Following operative correction of tetralogy of Fallot reticulocytes are diminished to a very low point and urobilin excretion is greatly increased. Both mechanisms a virtual cessation of blood formation and to an even greater extent blood destruction contribute to a reduction of the preoperative polycythemia.<sup>1</sup>

Lowered arterial oxygen saturation also prevails in patients with chronic pulmonary disease because of imperfect oxygenation of the blood in the lungs with resulting polycythemia as in patients with emphysema and fibrosis. Compensatory polycythemia occurs in persons living in areas at high altitudes where the partial pressure of oxygen is reduced and in those with methemoglobinemia and sulfhemoglobinemia in whom the oxygen-carrying capacity of the red blood cells is compromised by the presence of these pigments. The clinical manifestations of chronic mountain sickness which occurs in persons living at high altitudes resemble those of polycythemia.<sup>2</sup> Other causes of secondary polycythemia include brain tumors such as cerebellar hemangioblastomas, Cushing's syndrome, kidney tumors (particularly hypernephroma<sup>3</sup>), hydronephrosis<sup>4</sup> and often cobalt therapy.

Abnormally high erythrocyte values in the neonatal period in excess of normal for this period have been described in conjunction with anorexia and nervous system signs and symptoms.<sup>5</sup> Polycythemia also may occur as a complication of congenital adrenal hyperplasia<sup>6</sup> in the newborn infant in whom levels of hemoglobin and hematocrit may be so high as to require phlebotomies.

Boys and girls from 12 to 18 years of age often normally show maximal hemoglobin values of 16 to 18 gm per 100 ml and red cell counts of 5.5 to 6.5 million per cubic millimeter. These peak values simulating polycythemia recede in subsequent years.

## METHEMOGLOBINEMIA

Iron normally exists in the ferrous state in the iron porphyrin complex of the heme portion of the hemoglobin molecule. Methemoglobin is formed when hemoglobin in its deoxygenated state is oxidized to the ferric form. In this state iron cannot combine with oxygen and when methemoglobin is produced in significant amounts it reduces the oxygen combining capacity of the blood which is thus incapable of transporting oxygen. Methemoglobinemia is characterized by intense cyanosis due to the presence of methemoglobin in the circulating red blood cells in concentrations substantially above normal.

Primary or congenital and secondary or drug induced methemoglobinemia are the two major types of this condition. In patients with a less common form of congenital methemoglobinemia the defect lies in the globin component and is therefore included in the discussion of abnormal hemoglobins (hemoglobin M) (see Chapter 16).

**Pathogenesis.** Methemoglobin is being formed continuously in the red cells and is simultaneously reduced to hemoglobin by one or more enzymes in the erythrocytes so that the content of methemoglobin within the red cells is less

### ***Primary Polycythemia (Polycythemia Vera, Erythremia, Vaquez Osler Disease)***

Primary polycythemia is a specific disease of unknown etiology characterized by cerebral and cardiovascular symptoms a peculiar reddish purple color of the skin bloodshot eyes visual disturbances thrombosis and hemorrhage and usually splenomegaly. This disease occurs in middle and old age and is extremely rare in childhood.<sup>1-4</sup> The bone marrow is hyperplastic total blood cell formation is increased with a marked elevation of red cells hemoglobin level and hematocrit as well as of leukocytes and platelets. The total blood volume is strikingly increased. Intravenously administered radioactive phosphorus is the treatment of choice. The disease is chronic progressive and ultimately fatal. The absence of splenomegaly and particularly normal white cell and platelet counts rule out polycythemia vera.

**Benign Familial Polycythemia** Benign familial polycythemia is another type of primary polycythemia which occurs more frequently in children than does polycythemia vera. The patient one or more siblings and in some cases a parent manifest an elevated hemoglobin level (up to 27 gm per 100 ml) red cell count (10 million per cubic millimeter) and hematocrit (82 per cent) without evidence of underlying cardiopulmonary insufficiency. There is no splenomegaly or other physical signs except for a ruddy countenance and deeply injected conjunctivae. The red cell mass is increased but the leukocyte and platelet counts are normal thus differing from polycythemia vera.<sup>3,5</sup> No treatment is needed the prognosis is favorable.

### ***Secondary Polycythemia (Erythrocytosis, Erythrocythemia, Compensatory Polycythemia)***

The term secondary polycythemia signifies an increase in the total volume of red cells in response to a recognizable cause. Secondary polycythemia is most commonly found in infancy and childhood in patients with cyanotic congenital heart disease due to shunts from the pulmonary circuit. The mixture of unsaturated venous blood and arterial blood results in a lowered oxygen saturation. The hemoglobin ranges between 15 and 25 gm per 100 ml the red count between 5.5 and 9 million and the hematocrit between 65 and 85 per cent parallel with the red cell counts.<sup>1</sup> The mean red cell volume is usually higher than in normal children of corresponding age and weight ranging between 80 and 110 cubic microns.<sup>6</sup> The polycythemia serves as an extremely effective compensatory mechanism until the hematocrit reaches levels of 80 per cent or more. At this point the disadvantages of coexisting high blood viscosity outweigh the advantages of the increased available oxygen which polycythemia provides.<sup>6,7</sup> The response of the bone marrow to the persistent anoxic stimulus which accounts for the polycythemia is probably mediated by a humoral factor.<sup>10</sup> It is of interest that a hemoglobin concentration which is regarded as normal for the average child may represent a state of hypochromic anemia in the young patient with cyanotic heart disease and erythrocytosis. Iron therapy corrects the hypochromic anemia and is responsible for a further increase in the red cell count hemoglobin level and hematocrit. In the patient with polycythemia

discise The absence of signs of heart disease and spectroscopic examination of the blood reveal the diagnosis of methemoglobinemia

### ***Congenital Methemoglobinemia Associated With Hemoglobin M***

The defect in congenital methemoglobinemia associated with hemoglobin M lies in the globin component (see Chapter 16) Methemoglobin constitutes 15 to 25 per cent of the total hemoglobin in affected persons They live normal lives and are undisturbed by cyanosis Ascorbic acid and methylene blue exert no effect on the methemoglobin or cyanosis

### ***Drug Induced Methemoglobinemia***

Various compounds activate the oxidation of hemoglobin from the ferrous to the ferric state forming methemoglobin These direct oxidants include nitrites chlorates and quinones<sup>9</sup> and prominent among these compounds are aniline and its derivatives sulfanilamides acetamid phenacetin bismuth subnitrate and potassium chlorite In infancy and childhood the offenders are marking ink shoe dyes certain red wax crayons<sup>69</sup> well water containing nitrates from the soil meat containing a high nitrate content<sup>68</sup> and furniture polish containing nitrobenzene<sup>60</sup> Nitrate from contaminated water from shallow wells used in infant feeding mixtures is converted to nitrite in the bowel and on absorption causes methemoglobinemia<sup>1343</sup> The absorption of aniline dye from marking ink on diapers and other articles of infants clothing has been responsible for outbreaks of methemoglobinemia in nurseries which frequently affect premature infants<sup>3149387</sup> Similar poisoning from aniline dyes results from cutaneous absorption from freshly dyed shoes and blankets Methemoglobinemia has also been produced in young infants by the application of ointments containing benzo camphor<sup>84</sup> Intracorpuseular methemoglobin and sulphemoglobin have been noted in the course of hemolytic anemia caused by large doses of sulfonamides and toxic agents Heinz bodies and contracted red cells have been noted in the blood smears of affected patients

### ***Features Common to Methemoglobinemia***

**Diagnosis** Methemoglobinemia is suspected when there is a definite history of ingestion of or exposure to a toxic substance when cyanosis exists in the absence of evidence of cardiac or respiratory dysfunction The diagnosis is supported if a sample of venous blood retains the characteristic chocolate brown color after vigorous shaking with air for fifteen minutes Methemoglobin has a well defined absorption band spectroscopically at 630 m $\mu$  which disappears on the addition of 2 per cent solution of potassium cyanide A striking feature is the intensive peculiarly grayish cyanosis which develops within one to two hours after ingestion of the toxic substance The discoloration progresses rapidly until the skin and mucous membranes become almost black in color<sup>6</sup> In mild cases the child is fully conscious and in no distress In severe cases the patient has considerable anoxemia and dyspnea and develops circulatory failure

**Treatment** Patients with mild cases of acquired methemoglobinemia recover spontaneously upon withdrawal of the toxic substance In a patient with disease



than 2 per cent of the total hemoglobin.<sup>6</sup> The mean value for methemoglobin has been given as 2.2 per cent in premature infants between 1 and 15 per cent in newborn infants and infants in the first year of life and below 1 per cent thereafter.<sup>6</sup> The enzyme system responsible for normally reducing methemoglobin to hemoglobin is dependent upon the integrity of the erythrocyte and is associated with the glycolytic process.<sup>6</sup> The effects of the formation of methemoglobin are twofold. First methemoglobin as such becomes unavailable for transport of oxygen. Second the presence of methemoglobin renders the dissociation curve of the residual oxyhemoglobin less S shaped and more hyperbolic. Indeed this change causes a shift of the dissociation curve of the residual oxyhemoglobin to the left. The total effect is a lowered capacity for unloading oxygen to the tissues and hence a tissue susceptibility to anoxia.<sup>6-8</sup>

Relatively small amounts of methemoglobin approximating 15 per cent of the total hemoglobin are sufficient to produce cyanosis. The relative capacity of the pigments to produce cyanosis of comparable intensity has been given as 5 gm of reduced hemoglobin per 100 ml of blood, 15 gm per cent of methemoglobin and less than 0.5 per cent of sulphemoglobin.<sup>37</sup>

Cardiovascular mechanisms for increasing the oxygen supply to the tissues are called into play when the concentration of methemoglobin exceeds 40 per cent of the blood pigments. At levels exceeding 60 per cent ataxia, prostration and unconsciousness occur. Failure of an oxygen supply necessary for sustaining life could be expected to result when the methemoglobin concentration reaches about 85 per cent.<sup>8</sup>

Methemoglobinemia results clinically either from an inborn error of metabolism in which the intracellular enzymes normally reducing methemoglobin are deficient or from the effect of chemical or therapeutic agents which oxidize hemoglobin at a rate beyond the capacity for its reduction.

### ***Congenital (Familial) Methemoglobinemia***

Congenital (familial) methemoglobinemia is caused by a congenital absence of a reducing factor in the erythrocytes which is responsible for the reversion of methemoglobin to hemoglobin in normal red cells. The defect is often familial,<sup>38</sup> transmitted as a recessive. Patients with this disease suffer from a deficiency of the flavoprotein coenzyme factor 1 (diaphorase 1) which acts as a carrier in the conversion of methemoglobin to hemoglobin.<sup>44</sup> This enzyme deficiency results in an accumulation of methemoglobin in the red cells. The shift to the left of the oxygen dissociation curve is not always observed.<sup>40</sup> Without treatment the majority of these patients tend to reach equilibrium at about 40 per cent methemoglobin in the blood. Most patients show persistent diffuse cyanosis which is generalized but particularly marked in the fingers, toes, buccal mucous membranes, lips, nose, cheeks and conjunctiva. Clubbing of the fingers does not occur. There is no anemia but mild compensatory polycythemia may develop. Patients are usually asymptomatic except for occasional headaches and in older persons poor exercise tolerance may be noted. Usually there is no physical disability even with strenuous exercise. Life expectancy is unaffected. Congenital methemoglobinemia may be confused initially with cyanotic congenital heart

Cyanosis due to sulfhemoglobinemia which was both congenital and familial has been described in a newborn infant<sup>6</sup> In another case cyanosis due to sulfhemoglobinemia occurred in a 5 year old child with nonspecific gastroenteritis to whom a triple sulfonilamide had been given in the course of prolonged administration of a sulfur tonic<sup>35</sup>

## MISCELLANEOUS ANEMIAS

### *Anemia of Chronic Renal Insufficiency*

Anemia is a well known accompaniment of chronic renal failure irrespective of the type of lesion causing it It occurs in patients with primary diseases of the kidney such as acute and chronic glomerulonephritis chronic pyelonephritis and congenital polycystic kidneys or in those with diseases possessing renal components such as lupus erythematosus Within limits the degree of anemia varies with the level of nitrogen retention

**Pathogenesis** The two main factors concerned with the pathogenesis of chronic renal failure are the depression of erythropoiesis and increased red cell destruction Inadequate marrow response is evident from the poor utilization of intravenous injections of radioactive iron<sup>3, 36, 37</sup> The actual factor responsible for erythroid depression is still unknown but a toxic retention product remains a possibility There are indications also that kidney disease interferes with erythropoiesis by retarding the elaboration or activation of an erythropoietic stimulating factor produced by the normal kidney<sup>48, 49</sup>

Transfusion of the red cells of the patient or of a normal donor into the patient's circulation reveals a shortened life span for both types of cells<sup>1, 3, 38, 39</sup> The most marked hemolysis as judged by shortened red cell survival occurs during the period of increasing uremia and azotemia It is assumed that rapidly progressive anemia in the absence of detectable blood loss is caused by an extracorporeal hemolytic factor In patients with stationary chronic renal failure normal donor cells show no shortened survival Increased destruction of red cells in patients with chronic disease carries a poor prognosis Erythrocytes from patients with severe renal failure are normal as measured by their normal life span when injected into healthy recipients<sup>3</sup> indicating the absence of an intracorporeal defect It seems likely therefore that a combination of depressed erythropoiesis and increased cell destruction is manifest in some stage of chronic nephritis Blood loss from epistaxis and renal and gastrointestinal bleeding may contribute to the anemia since a hemorrhagic tendency is not uncommon in persons with chronic nephritis

**Laboratory Findings** The anemia is usually normochromic and normocytic occasionally it is microcytic or microcytic Contracted and deformed erythrocytes assuming a triangular shape have also been observed<sup>77</sup> Reticulocytes may be reduced in numbers<sup>11</sup> or slightly to moderately increased<sup>3</sup> the levels probably depending upon the degree of associated hemolysis Anemia occurs in practically all patients with any significant degree of azotemia<sup>11</sup> Hemoglobin levels average 8 to 9 gm per 100 ml in patients with moderate azotemia<sup>11</sup> but drop to 4 and 5 gm as nitrogen retention increases The white blood cells may be normal in number or slightly increased and there is little or no shift

of any degree of severity treatment should be initiated immediately. Methylene blue acts as a specific antidote converting methemoglobin to hemoglobin. The recommended dosage for infants is 2 mg per kilogram of body weight for older children 15 mg per kilogram and for adults 1 mg per kilogram. It is readily available in ampules of 1 per cent solution, the solution being given by slow intravenous injection. Treatment may be repeated if methemoglobinemia recurs. Ascorbic acid orally or parenterally also reduces methemoglobin, but the conversion takes place more slowly and therefore is not practical in urgent cases.

In patients with hereditary methemoglobinemia orally administered methylene blue or ascorbic acid may be given over prolonged periods to combat cyanosis and particularly associated symptoms such as headache. Ascorbic acid in an oral dosage of 500 mg given to a 12½ year old boy caused a significant drop in methemoglobin concentration.<sup>6</sup> Intravenous methylene blue acts more promptly but a gradual return to the original methemoglobinemia is inevitable when therapy is terminated.

### *Methemoglobinemia in Young Infants*

The susceptibility of young infants to the development of methemoglobinemia upon exposure to certain toxic agents has been shown to be due to a deficient reduction of methemoglobin in the red cells.<sup>6</sup> Well water containing a high percentage of nitrites causes methemoglobinemia in formula fed infants even though their parents drinking the same water remain normal.<sup>7,8</sup>

The differences in the reactions of the infants and their parents are explained by observations that the erythrocytes of cord blood reduce significantly less methemoglobin than do those of adult controls in the presence of lactate, lactate and methylene blue or glucose. The normal *in vivo* pathway for reduction of methemoglobin has been shown to be dependent upon the generation of reduced diphosphopyridine nucleotide (DPNH).<sup>44</sup> The red cells of the young infant have difficulty in reducing hemoglobin, presumably on the basis of a transient deficiency of either DPNH dependent hemoglobin reductase or of one of the enzymes responsible for its generation.<sup>70</sup>

### **SULFHEMOGLOBINEMIA**

Sulfhemoglobin is a pigment not normally present in the body. It is inert as an oxygen carrier. The combination of inorganic sulfides with hemoglobin *in vivo* results in its formation.<sup>71</sup> Sulfhemoglobinemia often accompanies methemoglobinemia. Once sulfhemoglobin is formed, it is stable and irreversible, disappearing after three to four months when the affected red cells are destroyed. In contrast to methemoglobin, methylene blue and ascorbic acid are of no value in converting sulfhemoglobin to hemoglobin. The absorption band of sulfhemoglobin is at 618 mμ and is unaltered by the addition of potassium cyanide solution.

Enterogenous cyanosis formerly of great interest refers to an ill defined clinical syndrome characterized by attacks of cyanosis, headache, abdominal pain and bowel dysfunction (either diarrhea or constipation). Sulfhemoglobinemia is frequently present, often associated with methemoglobinemia. In constipated patients sulfhemoglobinemia is attributed to the absorption of an enterogenous oxidizing agent such as hydrogen sulfide.

dences of intense blood destruction. Acute hemolytic anemias occur also in patients with selected viral infections such as virus pneumonia, infectious mononucleosis, influenza, and Coxsackie virus A infections<sup>22</sup>. In the large majority of patients however overt hemolysis rarely accompanies an acute infection and when it does occur suggests unusual susceptibility of the patient<sup>22</sup>. Slight degrees of increased blood destruction insufficient to give clinical manifestations are a more common occurrence. Aplastic anemia has also been known to occur in infants and children with severe infections.

Anemia due to chronic infections may be associated with a variety of disease conditions such as pneumonia, empyema, tuberculosis, rheumatic fever, osteomyelitis, pyelonephritis, wound infections, brucellosis, subacute bacterial endocarditis and intestinal parasites. Mild infections do not provoke anemia unless they are prolonged or recurrent. As a rule infections of less than a month's duration manifest no significant anemia.<sup>1</sup> Once anemia develops it gradually increases in severity with the progression of infection but eventually stabilizes.

**Pathogenesis.** Although the mechanism of the anemia of infection has not been completely elucidated there is convincing evidence that toxic depression of erythropoiesis and shortened survival of the red blood cells contribute to its pathogenesis.

It will be remembered that the heme portion of hemoglobin is a metal complex consisting of iron in the center of a porphyrin structure. The underlying porphyrin of the hemoglobin molecule is termed protoporphyrin. Coproporphyrin, uroporphyrin, and protoporphyrin represent the most important naturally occurring porphyrins. Coproporphyrin is usually the predominant porphyrin in the urine and feces. Human erythrocytes contain free coproporphyrin as well as free protoporphyrin. In patients with the anemia of chronic infection an excess of free protoporphyrin and coproporphyrin in the red cells and urinary coproporphyrin may be interpreted as an inability of the heme portion of the hemoglobin molecule to utilize synthesized porphyrins.

The decreased rate of the erythrocyte production is due to a quantitative defect in the conversion of protoporphyrin into hemoglobin. The increased amounts of free coproporphyrin and protoporphyrin in the red cells of patients with anemia of chronic infection are in agreement with this concept. The anemia of infection has also been regarded as a disturbance of iron as well as of porphyrin metabolism. In patients with infectious states iron is absorbed normally and leaves the plasma rapidly. Iron administered intravenously also increases the serum iron transiently being diverted to the tissues and thus rendered not readily available for hemoglobin formation.<sup>9</sup>

In addition to depression of erythropoiesis a hemolytic factor has been demonstrated as shown by a shortened red cell life span in patients with a variety of chronic infectious disorders.<sup>11</sup> A decreased red cell survival has been noted in children with acute rheumatic fever.<sup>23</sup> The fact that the bone marrow is unable to compensate for the moderate increase in the red cell destruction is further evidence that erythropoiesis is depressed.<sup>1</sup>

**Blood Findings and Other Laboratory Data.** The anemia due to chronic infection is rarely severe. The hemoglobin usually ranges between 8 and 11 gm per 100 ml with a corresponding reduction in the number of red cells

to the left of the oxygen dissociation curve. Platelets are present in normal numbers.

**Bone Marrow.** The bone marrow is normal or moderately hypercellular in the majority of patients but becomes mildly hyperplastic with excessive nitrogen retention.<sup>11</sup> In a series of thirty patients with uremia Gasser<sup>1</sup> found no relation between the degree of azotemia and the percentage of erythroblasts in the marrow. Both hypoplasia and hyperplasia of erythroid elements coexisted with all grades of azotemia. In five children with renal disease in whom erythroid elements of the bone marrow numbered less than 1 per cent four showed congenital malformations of the kidney. The remaining patient suffered from the acute hemolytic uremic syndrome<sup>11</sup> consisting of sudden severe hemolytic anemia (hemoglobin 2.6 gm per 100 ml), a positive Coombs test, renal failure (nonprotein nitrogen 137 to 222 mg per 100 ml), thrombocytopenia, hemorrhage and convulsions. Despite the clinical similarity to thrombotic thrombocytopenic purpura, there was no microscopic evidence of this disease. Bilateral renal cortical necrosis was found at postmortem examination.

**Diagnosis.** Chronic renal failure is to be considered in any child with prolonged and undiagnosed normochromic and normocytic anemia, especially when there is no response to iron therapy. Earlier in the course of the disease the urinary abnormalities may be minimal and easily overlooked and the blood pressure readings may be equivocal. In one such patient the sole radiologic defect was the presence of medullary cysts in otherwise normal sized kidneys.<sup>4</sup> Anemia and a rise in nitrogenous products will also be found in patients with congestive heart failure and impaired renal function. Temporary depression of renal function, azotemia and anemia also follow severe hemorrhage from the stomach and duodenum.<sup>6</sup>

**Treatment.** Since the severity of the anemia depends upon the degree and duration of nitrogen retention there can be no lasting improvement with any type of treatment unless renal function is improved. It does not respond to oral or parenteral iron, liver extract, folic acid or vitamin B<sub>12</sub>. Transfusion with packed cells remains the treatment of choice for symptomatic relief. Fresh blood is preferable because of a possible coexisting hemolytic element especially in the patient with advanced disease. The bone marrow may compensate to the point of stabilizing the hemoglobin so that transfusions are no longer necessary. Oral cobaltous chloride has been advocated in the treatment of anemia of chronic renal disease.<sup>10</sup> Although oral cobaltous chloride therapy temporarily increases erythropoiesis and hemoglobin synthesis, serious toxic complications have prevented its widespread clinical use.

### *Anemia of Infection*

With the advent of antibiotics and other modern therapeutic measures anemia due to infection has become relatively infrequent. Its incidence though small usually involves persons with chronic rather than acute infections. Severe anemias coexisting with infection are usually hemolytic in nature and have been observed in connection with sepsis due to streptococci, staphylococci and pneumococci. Sepsis in the newborn period for instance may provoke an anemia with evi-

an increase in the number of platelets. The anemia is normochromic and normocytic.

The number of reticulocytes increase on the second to the third day, reaching maximal levels from the fifth to seventh day and terminating by ten to fourteen days. The persistence of reticulocytosis is indicative of continued bleeding. Polychromatophilia and normoblasts appear with severe hemorrhage. During active regeneration there is a tendency toward macrocytosis. The increase in hemoglobin and red cells is often followed by a lag in hemoglobin synthesis due to a deficiency of building materials existing prior to the hemorrhagic episode. Blood production is proportional to the amount of iron available in the stores or after iron ingestion. The intensity of the stimulus is inversely proportional to the anemia depending upon normal bone marrow capacity.<sup>11</sup> In the absence of adequate iron stores the anemia becomes hypochromic and microcytic. Under favorable conditions and depending upon the size of the blood loss, normal values are reached in four to eight weeks. The bone marrow shows a normoblastic hyperplasia during the regenerative period. Alimentary azotemia occurs in patients with massive hemorrhage in the gastrointestinal tract frequently from a bleeding peptic ulcer.<sup>12</sup> The elevation of blood urea nitrogen is due to the absorption of digested hemoglobin.

**Clinical Features** Unless blood loss is rapid and extensive, there are few symptoms. With significant hemorrhage clinical signs are related to the fall in blood pressure and blood volume. Pallor, faintness, cold sweating, restlessness, tachycardia, rapid breathing, and shock are characteristic. In the newborn infant posthemorrhagic anemia with a rapid fall in hemoglobin, pallor, weakness, loss of muscle tone, and limpness are pronounced. Mild jaundice is noted when blood is sequestered in tissues and body cavities, as in the patient with a large cephalhematoma.

**Treatment** The immediate steps in treatment are the control of hemorrhage and restoration of blood volume to offset the reduction in blood pressure. Transfusions, preferably of whole blood, are indicated to restore both blood volume and the deficit of hemoglobin and erythrocytes. Less blood than the calculated amount lost (see Chapter 7) is given initially to avoid suddenly overtaxing the depleted cardiovascular system. Iron is prescribed for at least two months to secure optimal hemoglobin synthesis.

### **Chronic Hemorrhagic Anemia**

**Etiology** Chronic hemorrhagic anemia is due to repeated overt hemorrhages or concealed small hemorrhages frequently from the same sources responsible for a single massive hemorrhage previously described. The gastrointestinal tract is most commonly affected with bleeding from ulcerations, anomalies such as a Meckel's diverticulum, polyps, esophageal varices in portal hypertension at times associated with bleeding from hemorroidal vessels, bleeding into the lungs in idiopathic pulmonary hemosiderosis, coagulation disorders, severe epistaxes, idiopathic thrombocytopenic purpura, hookworm disease, and genitourinary bleeding.

**Clinical and Laboratory Features** Pallor, irritability, and anorexia occur

The anemia is usually of a normochromic normocytic type but when protracted it tends to become microcytic and hypochromic in which case slight anisocytosis and poikilocytosis are also present. Reticulocyte counts are normal or reduced. The serum bilirubin is normal and other evidences of blood destruction are lacking despite the shortened life span of the red cells. The bone marrow shows no hypoplasia; rather the cellularity is normal or increased. If the bone marrow is hyperplastic there is an increase in the granulocytic elements and at times of less mature erythroid elements.<sup>7</sup> The excessive granulopoiesis of the marrow often is manifested in the younger patient by marked leukocytosis which may reach the intensity of a leukemoid reaction often out of proportion to the nature or degree of infection. Hypoferremia is present; the serum iron is markedly lowered and the serum iron binding capacity is moderately reduced.<sup>13</sup> The serum copper, the free erythrocyte protoporphyrin and coproporphyrin and coproporphyrinuria are increased.

**Clinical Features** Pallor is indicative of the anemia but the predominant symptoms and physical signs are those of the basic infection. Chronic anemia in children especially when accompanied by an increased sedimentation rate requires a search for an underlying infection. The course and prognosis are determined by the nature of the causative infectious process rather than of the anemia.

**Treatment** The anemia is corrected with subsidence of the basic disease and the elimination of the focus of infection. Iron therapy is unnecessary for this substance is abundantly available in the tissues. Liver extract, vitamin B<sub>12</sub>, folic acid and other antianemic agents are without value. Increased erythropoiesis and hemoglobin synthesis have been reported with the administration of cobaltous chloride but undesirable side effects such as nausea, vomiting and goiter limit its usefulness. Blood transfusions are given for their supportive effects when the hemoglobin level drops below 8 gm per 100 ml especially in the event of debilitating signs and also when surgical intervention is contemplated.

### **Anemia of Acute Hemorrhage**

**Etiology** Acute blood loss may be primarily external or internal into the tissues, organs or body cavities. The severity of the anemia and symptomatology depend upon the amount and rate of blood loss. Anemia may result from trauma, hemophilia, thrombocytopenic purpura, sudden rupture of esophageal varices, portal hypertension and gastrointestinal ulceration in the course of leukemia and in the newborn infant from premature placental separation, rupture of a placental blood vessel, incision or rupture of the placenta itself and faulty tying, or clamping of the umbilical cord.

**Blood Picture** Immediately following the hemorrhage the blood volume is reduced and until it is restored the hemoglobin and red blood cell levels may be deceptively high because of vasoconstriction and stasis. During the next one to three days when the blood volume is restored by the entrance of tissue fluids into the circulation blood values decrease and give a true indication of the extent of blood loss. On the first day there is a transient polymorphonuclear leukocytosis with a shift to the left of these cells, the appearance of myelocytes and

covery from anemia was observed in a patient with severe hypochromic anemia and hemochromatosis following the administration of pyridoxine.<sup>76</sup> The disturbance in pyridoxine metabolism was unassociated with any disturbance in tryptophan metabolism. Intermittent pyridoxine deficiency has also been described in man with recurrent episodes of severe red cell hypochromasia, microcytosis, and a high serum iron.<sup>3</sup> These abnormalities were corrected by the administration of pyridoxine. It is of interest that the hematologic picture in pyridoxine deficiency closely simulates Cooley's anemia.

**Vitamin C and the Anemia of Scurvy** In the scorbutic infant the anemia is hypochromic and microcytic; in the adult it is normocytic or slightly macrocytic. Although anemia is a common finding in patients with scurvy, it has not been exactly determined in what manner a lack of vitamin C (ascorbic acid) interferes with hematopoiesis. In Chapter 13 on megaloblastic anemia it is shown that vitamin C plays a part in folic acid metabolism and that its deficiency contributes to the etiology of the anemia. Accelerated blood destruction also has been noted in patients with scurvy.<sup>77</sup> Contributing to the anemia is the loss of blood from hemorrhages characteristic of the disease. Anemia develops in scurvy in spite of adequate supplies of iron, vitamin B<sub>12</sub>, and folic acid.<sup>3</sup> The total white blood cell count, differential count, and platelet count are normal. Moderate leukopenia and thrombocytopenia are present in patients with scurvy. Treatment with ascorbic acid corrects the anemia promptly, resulting in an elevated reticulocyte count in four to six days.

### *Anemia of Hypothyroidism*

A mild normocytic or macrocytic normochromic anemia usually accompanies untreated hypothyroidism. The hemoglobin ranges between 9 and 11 gm per 100 ml, and the red cell count ranges between 3.5 and 4.5 million per cubic millimeter. The bone marrow is hypocellular.<sup>78</sup> Fatty bone marrow confined to the long bones has been described in persons with cretinism.<sup>7</sup> The yellow color of the skin in hypothyroidism is due to carotinemia—not to excessive bilirubin. The anemia is refractory to hematinics but responds to thyroid. Hypothyroidism may be superimposed on a pre-existing iron-deficiency anemia, in which case the anemia is hypochromic, microcytic, and additional treatment with iron is necessary.

Clinical and experimental evidence suggests that the thyroid has a definite influence in hematopoiesis.<sup>79</sup> Removal of the thyroid gland in rabbits results in a moderate and persistent anemia of the macrocytic type.<sup>80</sup> The anemia in persons with hypothyroidism may be regarded as an effect upon the bone marrow of the sluggish oxidation that occurs in all tissues.

### *Blood Changes in Lead Poisoning*

Lead intoxication except for industrial cases is relatively common in infants and children, with its highest incidence between the ages of 12 and 36 months.<sup>16</sup> Children are more susceptible to lead intoxication than adults. It is acquired from a variety of sources, such as repeated ingestion of chips of peeling lead paint from walls, plaster, window sills, frames repainted toys, furniture, and



with markedly lowered hemoglobin and red cell levels. The anemia varies with the duration and intensity of bleeding. The blood picture is typically that of an iron deficiency anemia. As iron stores are depleted a hypochromic microcytic anemia develops. Reticulocytes rarely exceed 5 per cent. Bone marrow activity is slightly increased to the extent of a mild to moderate normoblastic hyperplasia. The white blood count is normal. In patients with persistent anemia examination of the stools for occult blood is mandatory.

**Treatment** The source of bleeding must be localized. Iron is prescribed as for patients with nutritional iron deficiency anemia. Transfusions are given only exceptionally to patients in whom greatly lowered blood values have persisted over long periods and preoperatively to those being prepared for surgical eradication of a Meckel's diverticulum or other congenital anomalies.

### *Vitamin Deficiencies and Anemia*

Many of the vitamins especially those of the B group are concerned with some phase of erythropoiesis. Although this relationship can be demonstrated experimentally in animals only deficiencies of vitamin B<sub>12</sub> and folic acid have been definitely found to provoke anemia in man.

**Vitamin A** A severe anemia (hemoglobin 67 gm per 100 ml red cells 2710 000 per cubic millimeter) was noted in an allergic child whose diet contained no vitamin A supplement. The anemia and other symptoms of vitamin A deficiency promptly responded to large doses of vitamin A.<sup>4</sup>

**Riboflavin** A mild anemia has been observed in swine<sup>5</sup> and dogs<sup>6</sup> with riboflavin deficient diets.

**Nicotinic Acid (Niacin)** The relationship of nicotinic acid to anemia has not been established. Pellagra is associated with deficiency of nicotinic acid and other nutritional factors. Anemia is inconsistently associated with this disease. It can be microcytic, normocytic or macrocytic<sup>7,8</sup> and probably results from multiple nutritional deficiencies rather than from nicotinic acid deficiency per se. It is of interest that a patient with hypochromic anemia in the tropics who was refractory to iron therapy responded to nicotinic acid.<sup>10</sup>

**Pyridoxine (Vitamin B<sub>6</sub>)** Nutritional microcytic anemia due to pyridoxine deficiency has been produced in a number of animals notably in dogs and pigs.<sup>1</sup> The anemia is characterized by irregular reticulocytosis, normoblastemia, polychromatophilia, bone marrow hyperplasia, elevated plasma iron levels and hemosiderosis in the liver, spleen and bone marrow. The anemia fails to respond to iron or copper but is relieved by synthetic pyridoxine.<sup>11,12</sup> which also mobilizes the iron deposits from the tissues. Pyridoxine deficiency rarely occurs in man. However, in infant with severe hypochromic microcytic anemia induced by pyridoxine deprivation responded promptly to parenteral and oral administration of the vitamin.<sup>1</sup> Occasionally anemias refractory to the common anti-anemia agents have responded to pyridoxine. In one adult refractory hypochromic anemia and abnormalities in tryptophan metabolism were simultaneously corrected by the injection of pyridoxine.<sup>4</sup> In another patient with hypochromic anemia reported on from the tropics in whom there was no diarrhea or steatorrhea a satisfactory response was obtained with pyridoxine.<sup>10</sup> A moderate re-

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lead nipple shields and the inhalation of lead fumes from the burning of battery casings. The essential diagnostic features include dense bands at the ends of the long bones and metallic flecks in the large bowel observed on roentgenograms, increased content of lead in the urine (over 0.1 mg per liter in twenty-four hours) and blood (0.06 gm per 100 ml),<sup>17</sup> bluish black and stippled "lead lines" on the gingival margins, increased excretion of coproporphyrin in the urine, history of pica for paint and plaster, and definitive blood changes.

Among the important hematologic changes are basophilic stippling, moderate reticulocytosis, polychromatophilia, microcytic and hypochromic anemia, anisocytosis and poikilocytosis, and an increased number of target cells. The anemia is usually mild, but the hemoglobin level may range from 5 to 10 gm per 100 ml.<sup>18</sup> Anemia is brought about by the toxic effect of the lead upon hematopoiesis in the marrow and by an increased hemolysis of circulating red blood cells. In the marrow, lead injures nucleated red cells or precursors by interfering with normal maturation, resulting in defective hemoglobinization.<sup>19</sup> The disturbance in hemoglobin synthesis stems from the effect of lead in preventing the incorporation of iron in the protoporphyrin nucleus of heme, with resultant excretion of excessive coproporphyrin in the urine (essentially coproporphyrin III) and the accumulation of free protoporphyrin within the red cell.<sup>20</sup> This excess of free protoporphyrin in the erythrocytes accounts for their red fluorescence when thin wet preparations of blood of patients with lead poisoning are examined under ultraviolet light.<sup>21</sup> Several explanations have been given for the accelerated destruction of red cells in the peripheral blood. *In vitro* studies have shown that lead injures the surface of the red cell, rendering it inelastic, brittle, and susceptible to fragmentation by mechanical trauma, although red cell osmotic fragility is decreased.<sup>22</sup> The increase in stippled cells in the circulation following splenectomy results from the premature elimination of these defective cells by the spleen. The destruction of damaged red cells leads to bone marrow stimulation.

Stippling occurs in patients with many kinds of anemias but may be sufficiently pronounced in those with the mild form of Cooley's anemia to simulate lead poisoning. The basophilic granules, however, are often larger and coarser in persons with lead poisoning than in those with other anemias. The stippled cell has been interpreted as a reticulocyte in which the basophilic material has been altered by lead.<sup>23</sup> Stippling has also been ascribed to the injurious effect of lead on the ribonucleic acid of the young erythrocyte and its precipitation by Wright's stain to give the characteristic punctate basophilic appearance.<sup>24</sup> Many stippled red cells give a positive iron reaction.<sup>25</sup> The bone marrow shows erythroid hyperplasia and stippling of the red cell precursors. No constant changes have been observed in the total white blood cell count or platelet count.<sup>1</sup> Because iron deficiency may interdict or coexist with lead intoxication, iron therapy is required in addition to deleading measures.

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## Leukocytes—Cell Types

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The origin of white blood cells has been described elsewhere in conjunction with blood formation in the embryo (Chapter 1). These cells differ from the erythrocytes in the absence of hemoglobin and in the possession of a nucleus. They are larger than erythrocytes, ranging from 8 microns for the small lymphocytes to 15 microns for the largest monocytes. These colorless corpuscles constitute the most important elements in the body's defenses against invading microorganisms.

The leukocytes possess an extensive enzymatic apparatus whose specific uses have not been entirely explored. They contain enzymes such as glucuronidase, acid and alkaline phosphatase, and esterase, and biologically important substances of nonenzymatic nature such as glutathione, glucuronic acid, histamine, and glycogen.<sup>43</sup>

**Growth and Multiplication.** As in the red blood cells, growth and multiplication of the leukocytes are closely identified with the nucleic acid components of the cytoplasm and nucleus. Deoxyribonucleic acid (DNA) is found in the chromatin of the cell nucleus and ribonucleic acid (RNA) in the cell cytoplasm and nucleolus. These two substances can be differentiated by the cytochemical Feulgen test for deoxyribonucleic acid. Following mild hydrolysis with hydrochloric acid and treatment with reduced fuchsin, a red color is produced with the dye if deoxyribose is split off.<sup>44</sup>

Folic acid (pteroylglutamic acid—PGA) and folinic acid (citrovorum factor), its biologically more active form, are indispensable in the synthesis of deoxyribonucleic acid of the cell nucleus. In the treatment of patients with leukemia it will be seen that analogues of folic acid and of some of the nucleic acid components such as the purines act as antimetabolites. By gaining entrance into the cells, they block enzyme systems and interfere with their growth.

The functional demands of each of the white blood cells vary in relation to local tissue changes in health and disease. The white blood cells require a rich oxygen supply for growth and development in contrast to anoxic conditions required for optimal erythropoiesis.<sup>45</sup> When white blood cells are rapidly destroyed, as in patients with leukemia with excessively high white cell counts, large amounts of uric acid are liberated. In patients with acute leukemia being treated

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plasma. In the course of their disintegration cytoplasm which contains gamma globulin is released in the lymph and blood. The rate of lymphocyte dissolution has been attributed to hormonal control—more specifically through the medium of pituitary adrenocortical secretions.<sup>9, 1</sup>

Plasma cells like lymphocytes to which they are related are also associated with antibody formation. Evidences of this function are the increased numbers of plasma cells in persons with hyperglobulinemia, their deficiency in those with agammaglobulinemia, and the high concentration of ribonucleic acid in the cytoplasm. Severe impairment of immunologic response and decreased resistance to infection in  $\gamma$ globulinemia are associated with a sharp decrease of plasma cells.<sup>30</sup>

**Erythrophagocytosis.** Phagocytosis also plays an important role in the removal of damaged or altered erythrocytes from the peripheral blood. Polynuclear neutrophils and monocytes which have ingested intact red blood cells occasionally can be observed. They have been noted in patients with hemolytic disease of the newborn, infant idiopathic acquired hemolytic anemia, leukemia, promyelocytes,<sup>31</sup> chemical poisoning, paroxysmal cold hemoglobinuria, typhoid fever, and other infections. In children with acquired hemolytic anemia, marked erythrophagocytosis was observed when the buffy coat of the blood had been previously incubated at 37 C for one hour.<sup>34</sup> Erythrophagocytosis noted in the peripheral blood and in the spleen of patients with hemolytic anemia has also been related to the opsonic activity of autoantibodies altering the surface of the red cells.<sup>9</sup>

**LE Phenomenon.** Phagocytosis is also exemplified by the LE cell which constitutes an important diagnostic feature of disseminated lupus erythematosus, which is a febrile disease of unknown etiology characterized by malaise, fever, skin lesions, polyserositis, arthralgia, cardiac manifestations, and renal damage. A common feature is a biologically false positive serologic reaction for syphilis.<sup>34, 35, 36</sup> A palpable spleen and enlargement of superficial lymph nodes have been observed in an appreciable number of patients but are inconstant features. Of aid in diagnosis is the demonstration of LE cells from preparations of the blood and bone marrow.

A normochromic normocytic anemia with a hemolytic component is present in 3 to 5 per cent of patients. Leukopenia and an increased number of non-segmented neutrophils are seen in about one third of the patients at initial examination and increase subsequently to about one half at some time in the course of the disease.<sup>36</sup> Platelets may be reduced to the degree simulating thrombocytopenic purpura. With present methods of treatment including ACTH and steroids, patients are kept free of symptoms for a considerable length of time. Progressive renal failure constitutes the major problem.

An important diagnostic test for disseminated lupus erythematosus is the demonstration of the LE cell described by Hargraves.<sup>34</sup> The LE phenomenon is an *in vitro* reaction which depends upon the presence of a nucleolytic factor and active phagocytic cells that are attracted to the lysed nuclear material which it later engulfs.

The LE cell is a segmented neutrophil (rarely a monocyte) which has phagocytized an amorphous mass derived most often from the nucleus of poly-



with steroids for instance serious disturbances of kidney function may result

**Chemotactic Factors** The functional demands for each type of the white blood cells vary with local tissue changes. The stimulus for each type of cell is largely chemotactic. The direction toward which a cell migrates is influenced by the presence of foreign particles infecting organisms or substances elaborated from the site of tissue injury. This property is manifested chiefly by granulocytes, monocytes, and eosinophils but not by lymphocytes. The rapid disintegration of cells require a cycle of compensatory and regenerative mechanisms for their replacement. Under pathologic conditions such as pyogenic infections the nucleic acid and other products of tissue destruction serve as a stimulus for proliferation. A leukocytosis promoting factor which stimulates a discharge of granulocytes from the bone marrow has been isolated in inflammatory exudates. Other products produce leukopenia so that the leukocyte level observed at any one time depends upon the interaction of these opposing factors.<sup>42</sup>

The presence of another substance in normal plasma which expels granulocytic leukocytes from the bone marrow into the blood and to some of the organs has been described. This substance represents the expulsion factor which mediates the delivery of polymorphonuclear leukocytes to the circulation to replace those destroyed.<sup>41</sup>

Particularly in infants the bone marrow may produce sufficiently large numbers of myeloid cells to constitute a leukemoid blood picture in the course of a severe infection. In less severe infections in the younger patients abnormal responses may also occur producing a leukocytosis out of proportion to the stimulus.

The stimulus for production of a particular leukocyte varies with each type of cell and depends upon the nature of the chemical substances liberated from the affected tissues. Certain infections stimulate production of polymorphonuclear leukocytes whereas others may depress them.

**Functions—Phagocytosis and Antibody Formation** The polymorphonuclear leukocytes, monocytes, and other reticuloendothelial cells contribute to the body defenses by their motility and their capacity for ingesting and destroying invading bacteria and discharging granulocytes from the bone marrow.<sup>43</sup> Most of the white blood cells are capable of amoeboid movement and obey chemotactic stimuli. They play a part in the defense of the body by phagocytic and immunologic means. The ingestion of a particle by living cells is termed phagocytosis. Amoeboid mobility of the granulocytes and monocytes may be quantitatively evaluated and serves as an index of their viability.<sup>4</sup> Neonatal leukocytes obtained from cord blood have been found to have lower amoeboid and phagocytic activities when compared to maternal leukocytes and those of other adults.<sup>44</sup> This inferiority would indicate an inherent functional difference corresponding to that found in other organs and systems. The relationship to immunity in the neonatal period has still to be determined.

Macrophages refer to the monocytic cells which ingest not only bacteria but also large particles such as red blood cells. Lymphocytes possess slight phagocytic powers but participate more actively in the elaboration of antibodies.<sup>4</sup> These immune substances are associated with the gamma globulin of blood.

factor of the patient's plasma. There is evidence that the L.E. factor is a gamma globulin which acts as an antibody and combines directly with the cell nuclei and nuclear nucleoproteins.<sup>2, 31</sup>

That an immunologic mechanism may be responsible for the nucleophagocytosis of the L.E. phenomenon is suggested by the associated evidence of autoimmune hemolytic anemia, leukopenia and thrombocytopenia in this disease and by the experimental production *in vitro* of L.E. cells.

In other observations nucleophagocytosis produced experimentally by mixing antileukocytic serum with leukocytes from the same source resulted in structures resembling L.E. cells.<sup>32</sup> According to this hypothesis the L.E. plasma factor constitutes an "autoimmune" substance which stimulates antileukocyte antibody. In the presence of the L.E. factor white cells are sensitized and phagocytized by other leukocytes<sup>33</sup> and a high antibody serum would be responsible for both the ultimate development of L.E. cells and the formation of rosettes.<sup>34</sup>

Nucleolysis which is fundamental in the L.E. phenomenon must be distinguished from the nonspecific nucleophagocytosis. An example of the latter is the tart cell. This cell is occasionally found in normal bone marrow and in persons with pathologic conditions such as lymphoblastoma, multiple myeloma, pulmonary infection and metastatic carcinoma.<sup>35</sup> The distinguishing feature between the L.E. cell and the tart cell is the difference in the morphologic appearance of the respective inclusion bodies. In the L.E. cell the inclusion body is amorphous and is characterized by the absence of visible chromatin material. In the tart cell the engulfed material can be readily identified as a cell nucleus resembling most often the nucleus of a lymphocyte. The L.E. cell must therefore be differentiated from other cells of non-specific conditions in which an intact nucleus with a normal chromatin pattern is phagocytized. The significance of the tart cell is obscure. Hargraves<sup>36</sup> who first described both the L.E. cell and the tart cell emphasized the fact that the latter is usually a histiocyte whereas the former is almost always a neutrophilic polymorphonuclear leukocyte. Further more he stated that the inclusion body of the L.E. cell is usually a homogeneous purple staining mass with no visible chromatin pattern.

The engulfment of nuclei and nuclear fragments of other cells is commonly observed in specimens of heparinized bone marrow of normal persons and patients with conditions other than systemic lupus erythematosus.

Few positive results have been reported if these criteria are adhered to in patients with conditions other than systemic lupus erythematosus.<sup>37</sup> The L.E. cell, however, have been reported in patients with penicillin reaction, and the possibility of the association of this phenomenon with hypersensitivity has been suggested.<sup>38</sup>

L.E. cells should be sought for in patients with unexplained fever, leukopenia, thrombocytopenia, hemolytic anemia, arthritis, nephrotic syndrome and uremia in which the etiology is obscure.<sup>3</sup> In children protracted polyarthralgia, fever and leukopenia should lead to suspicion of disseminated lupus erythematosus and a search for L.E. cells. The characteristic scaly eruption of erythematosus over the bridge of the nose and extending to the malar prominences in a butterfly fashion may not always be conspicuous. It may reappear on exposure to sunlight and with the cessation of steroids and corticotropin therapy. Occasionally patients with autoimmune (acquired) hemolytic anemia and idiopathic thrombocytopenic purpura may develop overt manifestations of disseminated lupus erythematosus with positive L.E. tests following splenectomy.<sup>39</sup>

*Leukocyte Erythrocyte Test for Cytoplasmic Inclusion Methods of demonstrating L.E. cells and the tart cell without the use of anticoagulant have been devised. In each of these the patient's plasma is necessary element for producing L.E. cells; the L.E. factor, nuclear ma-*

morphonuclear leukocytes or lymphocytes. Swelling and loss of structure of chromatin material results with the lysed nuclear mass acting as a foreign body. This material is chemotactic and attracts phagocytic cells which engulf it. The cell usually, a neutrophil contains this nuclear mass and because of its size pushes the nucleus of the phagocytizing cell to one side thus only partially surrounding the inclusion body.

The ingested nuclear material varies in staining reaction from light pink to dark purple. It is stained by basic dyes and is Feulgen positive indicating its nuclear origin.

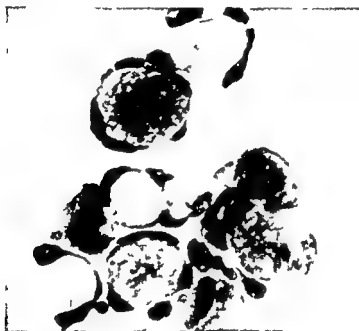


Fig. 29 LE cells. Polymorphonuclear cells (usually segmented neutrophils) containing homogenous nuclear material with nucleus displaced to one side ( $\times 1100$ ). (From Holman, Hulsted R. Systemic Lupus Erythematosus. *J. Pediat.* 56: 109, 1960.)

The LE rosette results from the attempt of a cluster of leukocytes to phagocytize simultaneously a single large mass of extracellular nuclear material. The presence of a plasma factor is indicated by the development of LE cells after incubating the plasma or serum of patients with systemic lupus erythematosus with the buffy layer from bone marrow or peripheral blood of normal persons.

The factor responsible for the LE phenomenon in patients with disseminated lupus erythematosus is associated with the gamma globulin fraction of the plasma. There are many explanations for the interaction between LE factors and the nucleus of cells. According to one theory the LE plasma factor induces specific chemical changes in leukocytic nuclei (namely the depolymerization of deoxyribonucleic acid) so that these altered nuclei are later ingested by other leukocytes to form LE cells. An alternate theory explains nucleophagocytosis on the basis of antinuclear autoantibodies which correspond to the LE serum

Wagner<sup>6</sup> demonstrated leukocyte-destroying factors which were active *in vivo* and *in vitro*. Drugs such as Pyrimidon combine with a protein in the serum forming an antigen which causes sensitization and antibody formation. The antibody becomes attached to the leukocytes which are agglutinated and destroyed when they come in contact with the antigen.<sup>7</sup> The serum of such a patient not only agglutinates normal human leukocytes *in vitro* but also produces a rapid fall of the white blood count when it is injected into a normal person. The destruction of agglutinated leukocytes occurs *in vivo* probably in the lung capillaries.<sup>1</sup>

Agglutination and destruction of circulating leukocytes have a wide application. This process has also been assumed to occur in persons with infections such as primary atypical pneumonia, cyclic agranulocytosis and pancytopenic states and in newborn infants with transitory granulocytopenia.<sup>14</sup> Bone marrow exhaustion follows peripheral depletion of injured or agglutinated granulocytes.

Leukocyte agglutinins may be detected by adding serum of an affected person to a suspension of leukocytes as completely free of red cells and platelets as possible.<sup>14</sup> The leukoagglutinin may manifest itself as an isantibody, an autoantibody or an allergic antibody in the patient with drug hypersensitivity. Since the serum substance destroys the patient's own leukocytes, an autoantibody analogous to the antibody against the red cells in patients with acquired hemolytic anemia appears to be involved. Fetal-maternal leukocyte incompatibility and the development of leukoagglutinins have been incriminated in infants with transfusion reactions and neonatal agranulocytosis.

## TYPES OF WHITE CELLS

The leukocytes present in the normal blood comprise three main groups: the granulocytic or myeloid series, the lymphocytes and the monocytes. The bone marrow and lymph nodes give rise to granulocytes and lymphocytes respectively. Monocytes originate from a reticular cell of the reticuloendothelial tissues present principally in the lymph nodes and spleen and to some extent in the bone marrow and other organs. The individual types of white cells differ from one another in structure and function.

### *Granulocytic or Myeloid Series*

For practical purposes, development of leukocytes may be regarded as proceeding in definitive lines: the myeloblast gives rise to myelocytes and granulocytes; the lymphoblast to lymphocytes; and monoblasts to monocytes. It has already been pointed out that primitive cells of both red and white cell series possess similar structural and staining characteristics and in their maturation reveal many common features. The immature forms of all white cells (the myeloblast, lymphoblast and monoblast) are not found in the peripheral blood of the normal person but their differentiation assumes importance in persons with leukemia in whom they infiltrate both bone marrow and blood.

**Myeloblast—Differentiation From Lymphoblast** In the patient with the common form of acute leukemia, differentiation is usually directed toward dis-

tional with which the LE factor can react and the phagocytic cell. Thus the use of white blood cells from blood or bone marrow of normal persons is eliminated. These tests are carried out with anticoagulants or with the use of clotted blood<sup>20</sup>. With the latter caution must be exercised in distinguishing tart cells (damaged nuclear material engulfed by phagocytic cells) from LE cells.

**Life Span of Leukocytes** The life span of the leukocytes has been divided into three phases: the hemopoietic phase extending from the development of the primitive cell to its delivery into the circulation; the intravascular phase, the period within the circulation; and the extravascular phase, the period of time the leukocyte spends in the viscera and in the tissues. The segment within the circulation therefore must be viewed in relation to the larger extravascular areas. The lung, liver, spleen, gastrointestinal tract, striated muscle, and kidney have been implicated as leukocyte removal sites.<sup>4</sup> The pulmonary circulation particularly represents a sizeable reservoir of leukocytes and platelets which may be readily discharged into the circulation under proper stimulation. The lungs are optimally located to deliver large quantities of leukocytes and platelets into the circulation more rapidly than are the bone marrow, spleen, liver, or other hemopoietic sites.

Various techniques have been used to estimate the life span of the white blood cells, but they have not yielded consistent results. A recent method<sup>6</sup> utilizes the incorporation of radioactive phosphorus into the deoxyribonucleic acid of the leukocytes. The majority of the labeled granulocytes enter the blood stream from the bone marrow at an age of six days and survive in the circulation for about nine days. By the same technique two groups of labeled lymphocytes were differentiated—one with a mean age of less than ten days and the majority with a mean age of about 100 to 200 days. The life span of eosinophils and basophils is approximately eight to twelve days.<sup>6</sup> The life span of three days or less has been given for the granulocyte series in patients with granulocytic leukemia<sup>21</sup> and about thirty days for the lymphocytes in patients with chronic lymphocytic leukemia,<sup>6</sup> both obtained by radioactive phosphorus labeling of the white blood cells with subsequent DNA extraction. In persons recovering from acute toxic leukopenia, approximately fourteen days may be required for maturation of primitive myeloblasts to the four- or five-lobed polymorphonuclear neutrophil.<sup>1</sup> The Pelger-Huet anomaly of the granulocytic leukocytes (poor segmentation and condensation of the nuclear chromatin described later on in this chapter) has been employed as a biologic tag to determine the survival time of the transfused neutrophils in the peripheral blood.<sup>22</sup> Most of the cells disappeared within six to eight hours and none were found after 49.5 hours. These results are comparable to those in a series<sup>4</sup> in which 80 to 85 per cent of the leukemic leukocytes tagged *in vivo* with a radioactive chromium were removed from the circulation within twenty-four hours. Some cells persisted for as long as five days.

**Leukoagglutinins** In persons with leukopenic syndromes, substances acting upon leukocytes have been detected which are comparable to the antibodies present in the serum of patients with thrombocytopenia and acquired hemolytic anemias. In the serum from a patient with agranulocytic angina Moeschlin and

**Promyelocytes (Progranulocytes) and Myelocytes** Promyelocytes are the same size or larger than myeloblasts. A nomenclature committee sponsored by the American Society of Clinical Pathologists and the American Medical Association<sup>2</sup> recommends the term progranulocyte for cells of the granulocytic series which have a nuclear structure too coarse for that of a blast cell and which have not developed discernible specific granules. This terminology has not been followed in the present text because it is difficult to identify this cell and because in the bulk of description the cell more mature than the myeloblast is the promyelocyte which does contain granules. The cytoplasm is deeply basophilic and may be abundant or confined to a narrow margin around the nucleus. The nuclei are round, the chromatin is coarser and the nucleoli are not so numerous or so sharply demarcated as those in the myeloblast. In contrast to the myeloblast which is devoid of granules, promyelocytes and myelocytes are granular. In the promyelocyte the granules are relatively few (the earliest forms have no more than ten granules), stain deep red to dark blue, and increase in number as the cell matures. A few granules overlie the nucleus.

The shift from promyelocyte to myelocyte is a gradual one and often difficult to separate into sharply defined categories. According to Sabins' classification, promyelocytes contain ten granules in early stages and a moderate number in a later stage, but the maximal numbers are not present until the myelocyte stage is reached. Furthermore, the granules in the promyelocyte cannot be differentiated into neutrophils, eosinophils, or basophils. Starting with the promyelocyte and continuing to the most mature granulocytes, the granules are oxidase positive and serve as an important feature in differentiating the granulocyte from the myeloblast.

Myelocytes are about the same size or smaller than the promyelocyte. The nuclei, which stain reddish purple, are large, round, oval, flattened on one side, or somewhat kidney shaped and are eccentrically placed. Nucleoli are indistinct and less numerous than those in the more primitive cells. Within the reddish purple cytoplasm are numerous dark granules which are scattered throughout and also cover the nucleus. As the myelocytes mature, the granules assume a definitive neutrophilic, eosinophilic, and basophilic character. Each of these cells in turn becomes a progenitor of the respective fully mature granulocytes.

Myelocytes frequently appear in the circulating blood of infants and young children in response to severe infection and hemolytic anemias marked by leukocytosis. Because of the more active response of the bone marrow in younger persons, the presence of small numbers of late myelocytes usually does not have the same connotation that it has in older persons. Occasionally, however, the leukocytosis and outpouring of young cells in children in response to infections may be sufficiently extreme to constitute a leukemoid reaction.

**Metamyelocyte or Juvenile Form** Metamyelocytes are smaller than the myelocyte, and the nucleus is oval and horseshoe shaped in older forms. The chromatin strands are coarse but not so deeply stained as those in more mature cells. The nuclear membrane is sharply defined as is that in the polymorphonuclear cell, and nucleoli are not observed. The cytoplasm is less basophilic than that

tinguishing between the myeloblast and the lymphoblast. Morphologic characterization however frequently does not permit definite classification into either category. Myeloblasts refer to any cell of the granulocytic series having fine chromatin structure and no specific granules. Usually nucleoli are visible. In patients with some forms of acute leukemia in whom sufficient numbers of promyelocytes justify the classification of the early forms as myeloblasts smaller primitive cells are found which are designated as micromyeloblasts. The latter seldom contain nucleoli but they can be distinguished from lymphocytes by their finely meshed chromatin.

Lymphoblasts may have a more sharply defined nuclear membrane, a coarser chromatin network and a smaller number of nucleoli than myeloblasts have but these features are not always detectable. The associated presence of substantial numbers of younger lymphocytes (prolymphocytes) and mature lymphocytes or promyelocytes and later myelocytes suggests a more definitive classification of the predominating immature cells as either lymphoblasts or myeloblasts. Both types of blast cells possess certain common fundamental diagnostic features. They are usually round, large (15 to 20 microns in diameter) and uniform in size. The narrow zone of the cytoplasm is basophilic and frequently vacuolated and the nuclear pattern is distinctive. Instead of masses of dense basichromatin such as are found in the mature cells the chromatin stains lightly and is finely granular and stippled or sievelike and shows the presence of nucleoli. In the absence of cytologic differences the primordial cell is often classified as a stem cell.

The morphologic differences between the myeloblast and lymphoblast when stained by Wright's method are listed in Table 14.<sup>7</sup> In acute leukemia of childhood the predominant blast cells usually favor the designation of lymphoblasts. Monoblasts are difficult to distinguish from myeloblasts and their diagnosis depends upon the presence of the more mature monocytes resembling them in the abundant granular gray blue cytoplasm and in the characteristic folded and indented nucleus.

*Table 14 Morphologic Differences Between the Myeloblast and Lymphoblast*

|                                    | Myeloblast                                      | Lymphoblast                                                                                            |
|------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Cytoplasm                          | Abundant                                        | Scanty                                                                                                 |
| Nuclear membrane                   | Smooth and thin                                 | Dense                                                                                                  |
| Chromatin                          | Fine network finely divided particles           | Coarse and some aggregation                                                                            |
| Nucleoli                           | 2 to 5                                          | 1 to 2                                                                                                 |
| Nucleolar membrane                 | Indefinite                                      | Distinct                                                                                               |
| Mitochondria (supravital staining) | Fine spherical scattered diffusely in cytoplasm | Larger ovoid thicker than myeloblast frequently clustered about nucleus or scattered through cytoplasm |

of the human polymorphonuclear neutrophilic leukocyte has been described<sup>14</sup> In the female a solitary "drumstick" with a well defined solid round head 15 microns in diameter is joined by a single fine chromatin strand to one of the main lobes of the nucleus These structures are rarely found in unsegmented forms or precursors This distinctive nuclear appendage which has been confined to the female has been found in persons of all ages and in blood specimens from the umbilical cord<sup>15</sup> These appendages are not to be confused with sessile nodules and related structures which are attached to the nucleus of the polymorphonuclear cell in both the male and female

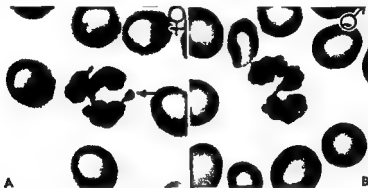


Fig 30 A Neutrophil in a blood film from a chromosomal female illustrating the accessory nuclear lobule that is present in an average of 2 to 3 per cent of neutrophils in females ( $\times 1800$ ) B A similar accessory nuclear lobule does not occur in neutrophils of chromosomal males ( $\times 1800$ ) (From Grumbach V M and Barr M L In Pincus G Recent Progress in Hormone Research vol 14 New York 1958 Academic Press Inc)

**Eosinophils** The eosinophil is a polymorphonuclear granulocyte the same size or a little larger than the neutrophil and possesses a bilobed or band formed nucleus The cytoplasmic granules are large coarse and spherical have an affinity for the eosin stain and take a bright reddish orange or deep red stain The granules are scattered through the cytoplasm and superimposed on the nucleus Eosinophils are less motile and more fragile than neutrophils They represent 1 to 4 per cent of the total number of leukocytes and possess a life span of six days<sup>16</sup>

**EOSINOPHILIA** Eosinophilia occurs in patients with several well defined groups of diseases in which there is an increase in eosinophils to about 5 per cent or 500 per cubic millimeter Following is a list of conditions in which eosinophilia occurs

- Allergic disorders
  - Bronchial asthma
  - Hay fever
  - Urticaria
- Skin diseases
  - Eczema
  - Psoriasis



in the myelocyte and in eosinophilic cast predominates. The granules are smaller stain less deeply and are clearly differentiated as neutrophilic eosinophilic and basophilic. Ameboid motion initially observed in the late myelocytes is definitely established in the metamyelocyte and characterizes the more mature cells to follow. The cytoplasm and nucleus may not develop evenly so that precise classification as to late myelocyte and metamyelocyte is not always possible.

**Polymorphonuclear Granulocytes** The mature granulocytes are described as polymorphonuclear cells because of the lobulation of the nucleus and are further classified as neutrophils eosinophils and basophils according to their reaction with the ordinary Romanowsky stains.

**Neutrophils** The polymorphonuclear neutrophil measures 9 to 12 microns with an average diameter of 10 microns. The cytoplasm is faintly pink and minute granules which fill the cell stain pink or violet. The nucleus consists of coarsely condensed chromatin strands which stain deep purple.

The nucleus is of two types—nonsegmented and segmented according to the absence or presence of lobulation. Segmented neutrophils which normally number 60 to 65 per cent of the white blood cells are mature cells in which the nucleus consists of two to five lobes connected by a thin strand of chromatin. The nonsegmented cells number 4 to 5 per cent of the total number of white cells and are variously designated as staff or stab cells. They are slightly smaller than metamyelocytes in which the nucleus fails to segment presumably due to toxic or other influences. Irregular condensation occurs in the nucleus of these cells with a pyknotic area at each end. Constriction of the nucleus in the middle or in other areas leads to odd shaped segmented forms. In the cells of persons with some infections the nucleus is short narrow bent on itself and deeply stained. Granulation in these cells is usually neutrophilic but may be eosinophilic or basophilic.

**"LEFT AND RIGHT SHIFTS (ARNETH COUNT COOKE AND SCHILLING COUNT)** According to Arneth's theory the nucleus of the polymorphonuclear cells becomes more segmented with age. The round or oval nucleus of the myelocyte becomes more indented as the cell matures with increasing segmentation in progressively older cells. As modified by Cooke and Schilling and in accordance with present usage a shift to the left implies the presence in the blood stream of immature forms consisting of large numbers of band forms. This shift is noted in infections toxic states and hemorrhage with the extent of the shift proportional to the extent of the disturbance. In patients with mongolism without infection there is a shift to the left in lobe count. In younger persons severe infections are often accompanied by the additional presence of myelocytes and metamyelocytes. The blood smear of patients with pernicious anemia sprue and megaloblastic anemia of infancy on the other hand demonstrate a shift to the right in which older neutrophilic polymorphonuclear cells predominate. In these conditions many of the granulocytes may be abnormally large and show hypersegmentation of their nuclei. Here too the lobes may vary in size and the chromatin is not so uniformly homogeneous as in normal cells.

**SEX DIFFERENCE IN NEUTROPHILS** A sex difference in the nuclear structure

of the human polymorphonuclear neutrophilic leukocyte has been described.<sup>14</sup> In the female a solitary "drumstick" with a well-defined solid round head 1.5 microns in diameter is joined by a single fine chromatin strand to one of the main lobes of the nucleus. These structures are rarely found in unsegmented forms or precursors. This distinctive nuclear appendage which has been confined to the female has been found in persons of all ages and in blood specimens from the umbilical cord.<sup>15</sup> These appendages are not to be confused with sessile nodules and related structures which are attached to the nucleus of the polymorphonuclear cell in both the male and female.



Fig. 30 A Neutrophil in a blood film from a chromosomal female illustrating the accessory nuclear lobule that is present in an average of 2 to 3 per cent of neutrophils in females ( $\times 1800$ ). B A similar accessory nuclear lobule does not occur in neutrophils of chromosomal males ( $\times 1800$ ). (From Grumbick M. M. and Barr M. L. In Pincus G. Recent Progress in Hormone Research vol. 14 New York 1958 Academic Press, Inc.)

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  - Bronchial asthma
  - Hay fever
  - Urticaria
- Skin diseases
  - Eczema
  - Psoriasis

## Skin disease—cont d

Scabies

Erythema neonatorum

## Parasitic infections

Trichinosis

Ascaris

Echinococcus disease

Visceral larva migrans

## Pulmonary eosinophilia

Eosinophilic pneumonitis (Löffler's syndrome)

Prolonged pulmonary eosinophilia

Tropical eosinophilia

Pulmonary eosinophilia with asthma

## Blood disorders

Eosinophilic leukemia

Hodgkin's disease

Recovery phase of acute infectious lymphocytosis

Postsplenectomy

## Irradiation

## Familial Eosinophilia

## Miscellaneous infections and disorders

Scarlet fever

Chorea

Drugs and chemicals

Benzol poisoning

Camphor phosphorus etc

Periarteritis nodosa

Metastatic neoplasms

From the time when it was first demonstrated that foreign materials such as parasitic extracts were chemotactic for eosinophils the relationship of these cells to diseases of sensitization has been amply confirmed. The number and proportion of eosinophils are greatly increased in patients afflicted with various types of allergy with parasitic infection especially by nematodes and following anaphylactic reactions. The eosinophil is the only cell which is known to respond specifically during states of hypersensitivity becoming attached to shock tissues.<sup>9</sup> The cell being phagocytic for foreign substances is a part of the body defenses.

A recently described condition observed in pediatric practice which is associated with eosinophilia is visceral larva migrans. This entity refers to infection with dog and cat ascarids (*Toxocara canis* and *Toxocara cati* respectively) and should be considered in the differential diagnosis of febrile illnesses associated with eosinophilia. A history of eating dirt or of exposure to dogs and cats in the home in patients with eosinophilia suggests visceral larva migrans associated with *Toxocara* infection. It represents an imperfect adaptation of the parasite to the host so that its life cycle in the latter is not completed. The eggs reach the soil through fecal contamination where within weeks they develop to infective stage larvae. If ingested by a child the larvae migrate through the intestinal wall via the vascular system and reach the liver, lungs, brain, eye and other organs. Instead of completing the migration back to the intestine as in the normal dog or cat host they either become encysted and are destroyed or continue to migrate through the tissues.<sup>8</sup>

Although liver biopsy may reveal characteristic larvae the diagnosis should be considered in the absence of this examination in young exposed patients with persistent or marked eosinophilia recurrent wheezing associated with pulmonary infiltration hepatomegaly of undetermined nature ocular disorders of obscure etiology unexplained central nervous system disturbances including convulsions and hyperglobulinemia.<sup>2</sup> In a patient with a similar condition there was an additional finding of a marked and persistent elevation of the heterophil agglutinin titer with antibodies similar to those found in persons with serum sickness.<sup>3</sup> Cases previously described as allergic hyperergic tissue responses resulting in disseminated visceral lesions<sup>4</sup> probably fit into this category as well.

Moderate eosinophilia with values of about 25 per cent can persist with fluctuations for a period of years in children without any identifiable cause except allergic symptoms. A syndrome of extreme leukocytosis and eosinophilia (up to 84 per cent) has been reported by Bass in which children who were chronically affected had a favorable outcome. When a normal leukocyte count has been restored a mild eosinophilia may persist. In the light of recent studies it is necessary to eliminate visceral larva migrans infection in patients with chronic eosinophilia.

Pulmonary involvement accompanied by eosinophilia occurs in a number of distinctive syndromes in which this combination exists. Löffler's syndrome the best known of these is a benign condition characterized by a patchy migratory infiltration which usually clears within a month. The accompanying eosinophilia reaches its peak in three to four days and disappears usually in ten to fifteen days. It is generally regarded as a response to an immune reaction taking place largely in the lungs. It has been suggested however that infiltrates in patients with Löffler's syndrome are due to the trapping of the eosinophils in the lung and not to an allergic pulmonary reaction.<sup>5</sup> It has been produced by infection with intestinal parasites plant products bacteria and drugs.

Prolonged pulmonary eosinophilia another syndrome described by Crofton and associates<sup>6</sup> is to be considered when radiographic shadows and eosinophilia persist for prolonged periods and are more widespread than in patients with Löffler's syndrome. It lasts two to six months and longer before recovery sets in and is marked by extreme eosinophilia. In many of these patients there is a personal or family history suggestive of an allergic diathesis. Probable etiologic factors include hypersensitivity to drugs and bacterial and parasitic infection.

Tropical eosinophilia or "eosinophilic lung" occurs in the inhabitants of India and many tropical countries. In persons with this syndrome there is some times an initial stage of malaise fever cough and dry cough the spleen may be palpable and eosinophilia is marked. The roentgenogram shows a diffuse mottling throughout both lung fields although sometimes the lesions are more localized. There may be an allergic background but more recently it has been ascribed to a mite infection of the upper respiratory tract.

Patients with pulmonary eosinophilia with asthma includes those in whom pulmonary infiltrations and eosinophilia are associated with symptoms incidental to chronic or recurrent bronchial asthma.

Patients with eosinophilic leukemia in contrast to those with other forms of eosinophilia show a marked relative and absolute increase in the circulating mature eosinophils which also infiltrate the tissues during the course of the disease. Patients with this type of leukemia respond for varying periods to ACTH and the steroid hormones.<sup>19</sup> They usually show no pulmonary changes of the magnitude described in patients with other conditions such as pulmonary eosinophilia except when an associated pneumonia occurs. Evans and Nesbitt reported on a patient who typifies others in whom the eosinophils showed a progressive immaturity as symptoms become more severe. Terminally a large proportion of the cells were myeloblasts in both blood and bone marrow. During this period however in which the only abnormality is confined to excessive numbers of mature eosinophils conditions other than leukemia are to be considered.

With the elimination of the major etiologic factors in causation cases of chronic eosinophilia in which the blood response persists for many years before the blood count eventually returns to normal are still encountered in children with poorly defined allergies.

**EOSINOPENIA.** Eosinopenia occurs following stress and has been related to the release of adrenocortical hormones. A prompt decrease occurs in circulating eosinophils in healthy persons following a single injection of adrenocorticotrophic hormone. The eosinophilic response must be interpreted as a nonspecific stress reaction of the intact organism. Eosinopenia occurs after administration of ACTH, epinephrine, surgical operations, infections, cardiac failure and any other conditions of stress.

The fall in the eosinophil count following injections of adrenocorticotrophic hormone (ACTH) has become widely used as a test of adrenal function. In the Thorn test<sup>23</sup> following the preinjection determination of the absolute eosinophil count 25 mg. of ACTH is given intramuscularly to the child or adult and the percentage decrease is measured in four hours. A reduction of 50 per cent or more in the eosinophil count is presumptive evidence that the cortical tissue has the normal capacity to respond to stimulation. A significant fall in eosinophils therefore does not occur in patients with chronic adrenal insufficiency such as Addison's disease.

No such clear cut results are obtained in the newborn infant. Apparently dosages ranging from 5 to 10 mg. are necessary to provoke eosinophilic responses beyond the range of spontaneous changes.<sup>21</sup> Farquhar<sup>24</sup> found a normal response in the newborn infant with a decrement of 30 per cent or greater when a 10 mg. dose was injected. On the basis of these observations the presence of physiologic transitory adrenal insufficiency in the newborn period depends upon the standardization of dosage. With adequate dosage the response of the adrenal cortex is found to be normal in the majority of newborn and premature babies in the first days of life.<sup>21</sup>

**Basophils.** Basophils originate in the bone marrow and are regular constituents of the peripheral blood. These cells are distinguished by the large round coarse bluish black and azurophilic granules which obscure the nucleus by their number. These granules are peroxidase negative and are situated above below

and at each side of the relatively lightly staining nucleus. The nucleus is round kidney shaped and slightly lobulated. The cytoplasm stains from pink to lilac. The basophil is motile and somewhat smaller than the mature neutrophil. The basophils number 0 to 0.5 per cent in children and adults. All stages of leukocytic differentiation are demonstrable in bone marrow for basophilic white blood cells. They are increased in patients with chronic myeloid leukemia, polycythemia, chronic hemolytic anemia, Hodgkin's disease, smallpox, chickenpox, some cases of cirrhosis of the liver after radiation and following splenectomy. In patients with lobar pneumonia, acute rheumatic fever or anaphylactoid purpura, the number of circulating basophils has been found to be low in the acute stage and high during recovery.

**Tissue Mast Cells (Tissue Basophils)** The tissue mast cell is also a granular basophil which shows little motility as compared with the active basophils. The separation of the tissue mast cells from the circulating basophils has been a subject of controversy.<sup>68,70</sup> Both have in common metachromasia of the granules. The cytoplasm of the mast cells is filled with granules which stain well with methylene blue, Wright-Giemsa stain or toluidine blue (1 per cent toluidine blue in methyl alcohol). Mast cells are found in groups about blood vessels and in the connective tissue where they originate.

Tissue mast cells, however, can be differentiated from blood basophils and are not interchangeable with them. Tissue mast cells do not possess myeloid precursors as do basophilic leukocytes. Functionally the two cells may have much in common in the production of several biologically active substances especially in the associated content of heparin and histamine in their granules.<sup>69</sup> Serotonin (5-hydroxytryptamine) has also been isolated from the tissue mast cells. There is suggestive evidence that serotonin and histamine act in conjunction to cause capillary permeability, hyperemia and edema which constitute the vascular response to acute inflammation.<sup>4</sup>

Morphologically tissue mast cells can be differentiated from basophilic leukocytes. The former possesses a normal vesicular nucleus which is rarely indented but the cytoplasmic contours vary widely, being round, irregularly oval, spindle or star shaped. The bluish cytoplasm may be hidden because of the heavily packed granules. These do not overlie the nucleus as they do in the basophil. By contrast the blood basophils usually have a small cell body with a polymorphic or lobulated nucleus typical of leukocytes and the granules tend to be irregularly distributed.<sup>6</sup> Accumulations of mast cells are observed in both the macular and papular type of urticaria pigmentosa, a dermatologic disorder occurring principally in childhood.

## Lymphocytes

Lymphocytes originate from lymphocytic tissue in many parts of the body, mainly from the lymph nodes, spleen, bone marrow, tonsils, thymus, gastrointestinal tract and liver. Considerable lymphocyte formation takes place in Peyer's patches. Lymphocytes enter the blood stream in two ways—indirectly through the thoracic duct, right lymph duct or other lymphatic-venous communications or directly through the walls of blood capillaries in lymphoid tissue. They

are actively motile cells whose function in antibody formation has already been discussed. They are classified into large and small cell types with the latter predominating. Large and intermediate forms of lymphocytes are assumed to be younger and less mature than small lymphocytes but this premise is to be established.

**Small Lymphocytes** Small lymphocytes vary in size from 6 to 10 microns in diameter usually about the same size as red blood cells. They contain a well defined round oval or indented nucleus with closely packed aggregates of chromatin which stains deep purple. The nucleus fills almost the entire cell with the periphery sharply defined and more heavily stained. The cells have a narrow rim of bluish cytoplasm which is often frayed and stains clear pale blue. Although the cytoplasm is usually devoid of granules in well stained preparations some of the cells contain a few distinct reddish purple or azurophilic granules of medium size. In thick smears the lymphocytes are spindle shaped with pointed projections of cytoplasm at opposite poles of the cell. This feature has no pathologic implications.

**Large Lymphocytes** Large lymphocytes vary from 10 to 15 microns in size. The nucleus is larger than that in the small lymphocyte, round slightly indented and thickened at the margins and the chromatin material is paler and not so markedly condensed as in the small lymphocyte. The cytoplasm is more abundant than in the small lymphocyte and frequently contains azurophilic granules and the edges are frequently scalloped and stain clear light blue. A perinuclear clear zone is a differentiating feature.

**Young Lymphocytes (Prolymphocytes)** Prolymphocytes are occasionally found in normal blood and more frequently in the blood of children especially those with chronic upper respiratory infections with associated tonsillar and cervical node enlargement. The cell is the same size as the large lymphocyte or is intermediate between the large cell and the small cell. The nucleus is less compact with light spaces between the chromatin threads. The most important feature is the deep blue basophilic staining of the cytoplasm which is homogeneous and agranular.

During the first months of life small numbers of lymphocytes which resemble the abnormal cells found in patients with infectious mononucleosis are occasionally observed in the blood smears from normal infants. These cells however bear no relationship to this disease or to any other related condition. Scattered vacuoles may be found normally in the rim of deeply staining blue cytoplasm. These cells are to be differentiated from the heavily vacuolated cells producing a foamy appearance characterizing the lymphocytes of infectious mononucleosis.

**Lymphoblasts** Lymphoblasts from which the lymphocyte is derived are characterized by a large round or oval nucleus staining reddish purple with fine granular chromatin which is slightly coarser than that found in the nucleus of the myeloblast. One or two nucleoli are present in the lymphoblast, the nuclear membrane is well defined and the cytoplasm is usually clear blue or deeply basophilic and nongranular.

## Monocytes

Monocytes measuring from 13 to 20 microns are larger than most cells found in the peripheral blood. They represent 5 to 10 per cent of the total circulating white blood cells. The monocyte is a motile cell with slow ameboid motion in contrast to the more active neutrophil. The cytoplasm is abundant in relation to the nucleus and stains darker than the pink cytoplasm of neutrophils. The pseudopods are blunt. The cytoplasm is a dull gray muddy blue color and is filled with large numbers of evenly spread fine lilac or reddish blue granules interspersed among which are a lesser number of unevenly distributed azurophilic granules and occasional vacuoles. The granules are peroxidase positive but in much lesser numbers and finer than those in the granulocytes. The gray blue cytoplasm of the monocyte contrasts with the clear light blue of the lymphocyte.

The nucleus is somewhat eccentric and possesses a skeinlike or lacy structure which stains lighter than that of the lymphocyte or metamyelocyte. It is a loosely arranged chromatin with light spaces and grooves in contrast to the clumped chromatin of the lymphocyte. The nucleus is indented, multilobulated and convoluted and often presents a folded appearance. The nuclear folds with the heavier staining at their margins are characteristic of the monocyte. The monocyte phagocytoses red cells, cellular fragments and the incompletely lysed nuclei of other cells as occurs in the formation of the tart cell.

**Young Monocyte (Promonocyte)** Although the nucleus of the mature monocyte may be round, it is more likely to be so in the young monocyte. The cell is somewhat larger and the cytoplasm is perhaps less granular and grayer than that in the older cell. The features are intermediate between those of the mature cell and the monoblasts.

**Monoblasts** Monoblasts are difficult to differentiate from the corresponding myeloblasts and promyelocytes. The cells possessing an irregular cell outline due to the presence of blunt pseudopodia are nonmotile. The cytoplasm is deeply basophilic with a grayish blue cast differing in this way from the blasts of other cell series. The cytoplasm contains dustlike reddish blue granules and vacuoles. The nucleus is large and round, often kidney shaped, horseshoe shaped or convoluted like the mature cell. The chromatin is fine and lacy and stains lighter than that in the mature monocyte. Nucleoli may or may not be present. The oxidase reaction is negative or poorly defined. The presence of monocytes and promonocytes in the same smear facilitates the diagnosis of the primitive monoblasts.

The Naegeli and Schilling types of acute monocytic leukemia<sup>24</sup> are described. In the Naegeli variety the myeloblast is regarded as the precursor of the monocyte so that the leukemia is myelomonocytic. The peripheral blood shows a predominance of monocytes in various stages of maturity in association with myeloblasts and young myelocytes. Many of the monocytic precursors may be difficult to distinguish from myelocytes. In the Naegeli type the monocytic cell also has a markedly distorted nucleus with a polymorphous shape; the entire cell is extremely large and the cytoplasm which is abundant is filled with fine purplish red granules smaller than the larger azure granules of the



myelocytes Scattered in the cytoplasm are small numbers of azure granules similar to those observed in the myelocyte

The Schilling type is a true monocytic leukemia in which the reticuloendothelial cells have been transformed into monocytes In this type the cells range from mature monocytes to monoblasts with only an occasional myelocyte Many of the primordial cells are difficult to differentiate from lymphoblasts or myeloblasts but the majority possess the characteristics of monoblasts In some of these cells and particularly in the promonocytes the nucleus is semitransparent lacy and folded on itself and the deeper underlying folds are clearly visible Promyelocytes and monocytes show a variable reaction with the peroxidase stain some negative and other weakly positive The Schilling type is often regarded as a variant of leukemic reticuloendotheliosis It is the less common of the two types of monocytic leukemia

**Monocytosis** Monocytosis occurs in the recovery phase of acute infection during the resolution process of pneumonia in the acute phase of rheumatic fever in active tuberculosis in Boeck's sarcoidosis in Hodgkin's disease in protozoal infections such as malaria in the recovery phase of agranulocytosis and in disorders of lipid metabolism A moderate increase in young monocytes may accompany the outpouring of the abnormal lymphocytes which characterize the blood picture in infectious mononucleosis Increased numbers of monocytes often occur in children with mesenteric adenitis accompanying upper respiratory infections in contrast to the polymuclear leukocytosis observed in those with appendicitis

**Monocytes in Tuberculosis—Monocyte Lymphocyte Ratio** Sabin and co workers<sup>9</sup> demonstrated that the major effect of the tubercle bacillus was on the monocyte of the connective tissues and blood The epithelioid cell of the tubercle has been proved to be an altered phase in the life cycle of the monocyte According to this view the lipoids of the tubercle bacillus are solely responsible for the monocyte epithelioid proliferation and differentiation in patients with tuberculosis<sup>9,1</sup> Whereas the tubercle bacillus grows and multiplies within the monocyte lymphocytes are intimately connected with limiting the spread of the infection In patients with tuberculosis the ratio of monocytes to lymphocytes in absolute numbers has been used to express the extent of invasion as compared to the degree of resistance An increase in monocytes with an increase of the ratio of monocytes to lymphocytes indicates a serious prognostic sign a lowering of the ratio with an increase in lymphocytes indicates a favorable sign<sup>9</sup>

Others have observed that the changes in the monocyte lymphocyte ratio are caused more often by a consistent decrease in lymphocytes during active disease and increase during healing than by the more irregular fluctuations of the monocytes According to this view a sounder interpretation of the clinical status of the tuberculosis process can be made by evaluating each cell type separately especially the lymphocyte than by calculating the monocyte lymphocyte ratio<sup>1</sup>

### Miscellaneous

**Histiocytes** Histiocytes are occasionally seen in the peripheral blood and in the bone marrow They are derivatives of the reticuloendothelial system and are



## DEGENERATIVE AND TOXIC CYTOPLASMIC CHANGES

**Amato Bodies** Amato bodies are irregular pale blue staining cytoplasmic clumps up to 2 microns in size. They occur in polymorphonuclear neutrophils in patients with various infections such as diphtheria, scarlet fever, pneumonia, and other coccal conditions and have been mistaken for inclusion bodies possibly of etiologic significance. Amato bodies are probably the same as Dohle bodies (see p. 338).

**Toxic Granules** Toxic granules and vacuolization are also regarded as degenerative changes occurring in the cytoplasm during acute infections. Excess vacuolization may be especially noticeable in patients with severe infections and in the blast cells of patients with leukemia. The toxic granules are deeply staining irregularly distributed coarse basophilic granules that are optically negative and occur in segmented and nonsegmented polymorphonuclear neutrophils. They have been shown to be liquid droplets and not granules and represent a leukocyte that has undergone cloudy swelling.<sup>23</sup>

**Pelger Huet Phenomenon of Granulocytes** Pelger Huet phenomenon of granulocytes is a hereditary anomaly characterized by partial or complete failure of segmentation of polymorphonuclear cells so that the number of nonsegmented cells is enormously increased. The nucleus is usually broad and kidney shaped and sharply delimited, consisting of strands of intensely stained chromatin with the intervening spaces clear. The conglomerates are particularly prominent in the blunt end of the nucleus. Segmented cells with two and sometimes three lobes occur less frequently, but the clumping and condensation of chromatin are present in these cells as well. The presence of the nonsegmented cells here does not represent a shift to the left as occurs in patients with infections and usually has no effect on health. This anomaly is transmitted as a non sex linked mendelian dominant character.<sup>24</sup> An acquired form has been described in an adult with chronic myelogenous leukemia.<sup>1</sup> The Pelger Huet anomaly has also been described as an acquired form in other myelopathies such as acute leukemia and myeloid metaplasia especially following prolonged exposure to myelotoxic therapeutic agents.<sup>10a</sup>

**Russell Bodies** The cytoplasm of a plasma cell may contain prominent spherical hyaline bodies or globules which take an acidophilic stain. These masses termed Russell bodies occur singly or in groups and are regarded as consisting of mucoprotein secreted by the parent cell. They appear in the plasma cell in myeloma and in plasma cell leukemia.<sup>6</sup>

**Hematogones** Lymphocyte like cells termed hematogones regarded as blood cell precursors are occasionally encountered in the bone marrow rarely in the peripheral blood. In contrast to the large erythrogones (hemocytoblast) these cells resemble small lymphocytes in size and morphology. They differ from the lymphocytes to the extent that they possess a denser more homogeneous slightly purplish dull matlike nucleus either with a narrow rim of cytoplasm or entirely devoid of it. Occasionally the nucleus is partially or completely traversed by a fine cleft. The hematogones often present the appearance of naked nuclei and also differ from the nucleus of the normoblasts in their lighter staining. These cells are found occasionally in the blood of young infants but more frequently

singly or in groups. These cytoplasmic inclusions are known as Russell bodies and consist of mucoprotein.

Plasma cells are regarded by some as an independent series and by others as a form of lymphocyte.<sup>4</sup> In either case the mature cells are derived from plasma blasts with intermediate forms constituting young plasma cells. Plasma cells rarely appear normally in the peripheral blood but are present in the bone marrow, lymph nodes, spleen and other areas. They are found in greatly increased numbers in the peripheral blood in patients with multiple myeloma, plasma cell leukemia and to a lesser extent measles, rubella, chickenpox, serum reactions and skin disorders. The so-called myeloma cell is a derivative of the early plasma cell and shows the main features of the plasmablasts.

It seems well established that plasma cells are primarily responsible for the synthesis of the antibody components of gamma globulin, perhaps to a greater extent than the lymphoid elements. In patients with agammaglobulinemia there is a failure of plasma cell proliferation. In biopsy specimens of lymph nodes in these patients no plasma cells or secondary follicle formation appears in response to injections of a bacterial vaccine and no antibody is formed.<sup>40</sup> Good<sup>31</sup> failed to find plasma cells in the bone marrow of patients with agammaglobulinemia after infection and after the antigenic stimulation as occur in normal children.

On the other hand, hypergammaglobulinemia is most often associated with an increase of plasma cells in the lymph nodes, spleen and bone marrow. The concept that antibody and gamma globulin are produced by plasmacytes is given further support by the close correlation observed between the plasmacytic development in the bone marrow and increased gamma globulin production in patients suffering from acute rheumatic fever and in patients convalescent from streptococcal pharyngitis.<sup>3</sup> Abnormal protein formation and marked hyperglobulinemia occur in patients with plasmacytic malignancies such as multiple myeloma and plasma cell leukemia.

**Türk's Cell (Türk Irritation Cell)** Türk cells are the same size as plasma cells and are closely related to them. They may be identical with the plasma cell, an intermediate cell, or represent an atypical lymphocyte. The cytoplasm is non-granular and stains deeper blue than the plasma cell, and the nucleus is eccentric but the chromatin is not cartwheel shaped. These cells are increased in number in the peripheral blood of patients with severe anemias, measles, rubella, agranulocytosis and chronic infections, especially when associated with a marked leukocytosis.

**Rieder Cells** Rieder cells are myeloblasts in which the nucleus is deeply indented, resembling lobulation. This conformation has been ascribed to the more rapid maturation of the nucleus than the cytoplasm.

**Auer Bodies** Auer bodies or rods are small or elongated slender rods which stain red-purple or azurophilic with Wright's stain and are peroxidase positive. They are sometimes found singly or in larger numbers in the cytoplasm of myeloblasts, myelocytes, monoblasts, monocytes and granular histiocytes (Ferrati cells) but not in the cytoplasm of lymphocytes or lymphoblasts. Most commonly cells which contain Auer bodies are undifferentiated without granules in the cytoplasm and their presence in such cells is suggestive of leukemia.

leukocytes in conjunction with abnormally formed or pyknotic nuclei. The inclusions are usually larger and fewer in number than other types. In one report three to six large irregular slate green masses were noted in the cytoplasm of the polynuclear neutrophils. In some cells they were smaller and more numerous. These inclusion bodies have been described in patients with malignant lymphoma and in conjunction with a hereditary pattern marked by semialbinism, photophobia to direct light, excessive sweating, pale optic fundi, hepatosplenomegaly and generalized lymphadenopathy.<sup>7, 18</sup>

**Reilly Bodies** In patients with gargoylism (Hurler's syndrome, lipochondrodystrophy) a rare disease of early childhood characterized by dull, coarse, cretinoid facies, dwarfism, skeletal deformities, clouding of the corneas and mental retardation, fine and coarse blue colored granules have been described by Reilly in some 60 to 90 per cent of granulocytes and their precursors in the bone marrow and occasionally in the lymphocytes and monocytes.<sup>69, 70</sup> These granules are the same as those noted by Alder as a hereditary anomaly. Patients with gargoylism have been described in whom the abnormal inclusions were confined to the lymphocytes, the granulocytes being unaffected.

## CELL STAINS

**Romanowsky Stains** Romanowsky stains of which Wright's stain is a modification consist of a combination of methylene blue with eosin. Depending upon their acid and basic affinities the red and blue structures of cells are identified in contrasting colors. The nuclei of white cells stain purplish blue with clear separation of basichromatin and oxychromatin; neutrophil granules stain light pink or lavender and the granules of eosinophils and basophils stain red and deeply azurophilic respectively.

**Supravital Staining** Supravital staining<sup>19</sup> permits the examination of cytoplasmic structures of blood cells in the living motile state. In the combination employed the mitochondria are stained by Janus green and the specific granules and vacuoles of the cytoplasm by neutral red dye. The nucleus and cytoplasm are left unstained but the nuclear outlines are readily discerned. Mitochondria are most plentiful in the blast cells and decrease progressively as the cells mature, being reduced in the late myelocytes and frequently absent in the mature polymorphonuclear leukocyte, lymphocyte and monocyte.

At times the blue green stain may serve in differentiating the blast cells by the shape and size of the mitochondria and their location. In the myeloblast the mitochondria are numerous and extremely fine and are scattered diffusely or packed in a segment of the cytoplasm. In the lymphoblast they are short, thick, oval or spherical and may be clustered around the nucleus or may be scattered diffusely. In the monoblast they exist as fine, slender rods scattered through the cell.

Motility is absent in the blast forms but is present in varying degrees of amboid motion in the mature cells of the three series, most marked in the polymorphonuclear neutrophil. In the monocyte the supravital stain is particularly diagnostic since the neutral red bodies are characteristically clustered as a rosette in the indentation of the nucleus. Supravital staining does not replace fixed smear

in the bone marrow in patients with pure red cell (chronic aregenerative) anemia<sup>77</sup> lymphocytic leukemia and more particularly giant follicular lymphoblastoma in whom increased numbers have been observed in both the peripheral blood and bone marrow<sup>71</sup> The hematogone may be the same cell included in the classification of micromyeloblasts undifferentiated primordial cells or hemocytoblasts

**Miscellaneous Inclusion Bodies** Atypical granules or inclusion bodies have been described in all types of white blood cells especially in granulocytes

**Alder's Anomaly** Coarse dark azurophilic granules in the cytoplasm of the white cells especially the neutrophils occur as a rare hereditary anomaly without pathologic significance<sup>41</sup>

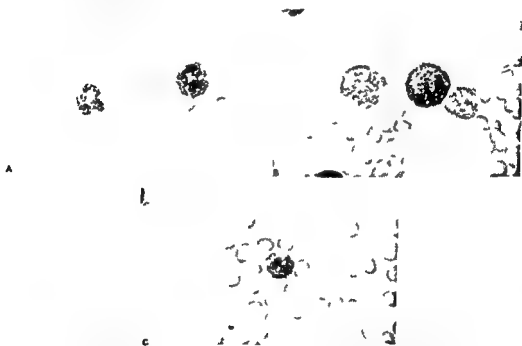


FIG. 31 Blood and bone marrow smears from a boy 3 years old with Hurler's syndrome. Heavy granulation (Reilly bodies) present in polymorphonuclear leukocytes of the peripheral blood. A myelocytes of the bone marrow. B and lymphocytes C are characteristic of this disease. These granules differ from toxic granulation and those found in the basophilic leukocytes. Although coarse granules are common fine and medium granules may also be present (Courtesy Dr. Ralph L. Engle, Jr., New York, N. Y.)

**Dohle Bodies** Round or oval shaped bodies of bluish cytoplasm either just visible or ranging in diameter to approximately 1 to 2 microns have been described in the cytoplasm of the polymorphonuclear neutrophil cells in patients with scarlet fever and other specific infections. They appear during the first day or two after a burn and often disappear when the skin is nearly completely covered.<sup>89</sup> Amato bodies are probably the same as Dohle bodies (see p. 337).

**Chediak's Anomaly of Leukocytes** These are Dohle-like azurophilic granules ranging in size to 2 to 5 microns in diameter in the cytoplasm in all types of

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but serves as an adjunct in the differentiation of cells and as an aid in visualizing their physiologic activity

**Peroxidase Stain** The presence of an oxidizing ferment in the cytoplasm of myeloid cells provides an added means of differentiating these from other cells. In the commonly used techniques (such as the Goodpasture and the copper peroxidase method of Sato and Sakai) this ferment causes the oxidation and precipitation of benzidine by hydrogen peroxide. Cells of the granulocytic series including promyelocytes give a strong peroxidase reaction as contrasted with lymphocytes plasma cells and red cells which are peroxidase negative. Monocytes show fewer and less well defined peroxidase positive granules than do granulocytes. Myeloblasts give a negative reaction hence this reaction is of no value in separating these cells from lymphoblasts.

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## *Leukopenia and Leukopenic Syndromes*

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*Leukopenia* denotes the condition in which the number of circulating white blood cells is below 4000 per cubic millimeter without reference to the type of leukocyte involved. Except when the leukocyte count is exceedingly low and all types of white blood cells are reduced, leukopenia is usually due to a reduction in the neutrophils. Infrequently the lymphocytes are markedly reduced, resulting in lymphocytosis.<sup>2</sup> On the other hand, in some instances there may be neutropenia without leukopenia. This is seen occasionally in serial white blood counts of patients with many conditions of protracted leukopenia, although the granulocytes are consistently reduced. In such an event the bulk of cells are made up of lymphocytes occasionally with monocytosis.

*Pathogenesis.* Leukopenia is brought about by a variety of mechanisms.<sup>1</sup> These include diminished manufacture of white blood cells as a result of simple inhibition (for example overwhelming infection, septicemia),<sup>3</sup> maturation arrest (for instance agranulocytosis), aplasia of the marrow (for example aplastic anemia), damage or destruction of leukocyte forming tissue (such as that caused by chemical and physical agents), destruction or inhibitory effect of the spleen on granulocytes (that is splenic neutropenia), or direct action on the peripheral blood by leukocyte agglutinins. A redistribution of the white cells conceivably also may cause peripheral leukopenia by withdrawing granulocytes from vascular channels to localized areas of pus formation. Replacement of the bone marrow by leukemic or neoplastic cells or by cells of entities such as Gaucher's or Hodgkin's disease results in leukopenia in some phase of the respective diseases. The course is unknown but has been attributed to crowding of myeloid tissue or a disturbance of local metabolism by the rapid multiplication of invading cells. In some cases the concept of hypersplenism is involved.

Leukocyte agglutinating factors responsible for the production of agranulocytosis and leukopenia have been demonstrated in the serum of patients. The formation of antibodies capable of agglutinating white blood cells in the blood stream has led to the concept of immunogranulocytosis or immunoleukopenia. This phenomenon is comparable to autoimmune mechanisms involved in the produc-

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**Infections** A persistent leukopenia may indicate a serious prognosis in the presence of certain infections in which leukocytosis ordinarily is expected.<sup>29</sup> Its presence is therefore of diagnostic value in patients with bacterial infections such as typhoid, paratyphoid, and undulant fever, with certain virus diseases such as influenza, measles, rubella, and primary atypical pneumonia, with protozoal infections such as malaria, and with the early stage of rickettsialpox.

Leukopenia is noted in about one third of patients with disseminated lupus erythematosus on initial examination and develops subsequently in about one half sometime in the course of the disease.<sup>1</sup> A moderate increase in nonsegmented neutrophils is seen in most patients.

**Chemicals, Drugs, and Irradiation** Chemicals and drugs such as anticonvulsants, antimicrobial agents (that is, chloramphenicol), antithyroid drugs (that is, thiouracil and its derivatives), tranquilizers,<sup>30</sup> antihistamines (that is, Pyribenzamine) and sulfonamides are occasionally associated with leukopenia and neutropenia. Exposure to physical agents such as irradiation and to chemicals such as antimetabolites, nitrogen mustards, Myleran, and 6-mercaptopurine, which are employed in the therapy of leukemia and neoplastic disease, can be expected to produce leukopenia and a hypoplastic bone marrow when given in sufficient dosage and over a variable length of time. Depending upon individual susceptibility and duration of exposure, benzol results in leukopenia, agranulocytosis, and a complete picture of aplastic anemia. In hypersensitive persons, aminopyrine and related antipyretics may cause serious and even fatal leukopenia and agranulocytosis.

Not only do these chemicals exert a destructive effect upon myelopoietic cells in the bone marrow, but also, as stated previously, with regard to drug-sensitive persons, agglutinins can account for peripheral destruction of leukocytes. According to this concept, bone marrow depletion and exhaustion result secondarily from the accelerated destruction of granulocytes in the peripheral blood.

The lymphocyte is the most radiosensitive of all white blood cells, and the most important change in the white blood cell picture after exposure to radiation is a reduction in the total number of these cells. This is principally due to a decrease in the number of granulocytes which numerically represent the majority of blood cells in the average human being.<sup>13</sup>

**Miscellaneous** The miscellaneous causes of leukopenia include a wide variety of conditions.

**AGRANULOCYTOSIS** The incidence of fatal agranulocytosis is greater in adults than in children. Secondary neutropenia may occur following the administration of drugs or following infections which damage the bone marrow. Although the disease occurs primarily in adults, greater heed should be given this condition in childhood because of the increasing use of drugs and antimicrobial and antibiotic agents.

Agranulocytosis is an acute febrile disease with a high mortality rate characterized by leukopenia, pronounced reduction in neutrophils, and slight if any changes in the red blood cells or platelets. An important clinical feature is the necrotic lesions in the throat and elsewhere. Most cases of agranulocytosis are attributed to the effect of one of a number of drugs in a susceptible person.

tion of certain types of idiopathic hemolytic anemia and thrombopenic purpura. Antileukocyte activity has been demonstrated in the serums from patients with a wide variety of hematologic diseases with leukopenia especially in those with a hyperplastic marrow.<sup>30</sup> The majority of these agglutinins are blood group specific and a small percentage are nonspecific (globulins).<sup>18</sup> This agglutinating mechanism has been applied to the leukopenic effect of drugs and chemicals<sup>31</sup> to idiopathic immunoneutropenia<sup>1</sup> and to the development of the L.E. phenomenon in disseminated lupus erythematosus.<sup>32</sup> Transitory granulocytopenia of the newborn infant (neonatal agranulocytosis) has also been explained on the basis of immunoleukopenia.<sup>33</sup> Leukoagglutinins occur more frequently among persons who have had transfusions especially repeated transfusions—hence the possibility that they represent antibodies to antigens of leukocytes.<sup>3</sup>

**Bone Marrow** Although the bone marrow reflects the state of granulopoiesis the bone marrow findings cannot always be correlated with those in the peripheral blood. The combined examination is necessary however for interpretation and management of a leukopenic condition.

The bone marrow ranges from the normal to a maturation arrest at the promyelocytic level. The variability depends upon the stage, duration and severity of the disease when the bone marrow is examined. Thus the peripheral leukopenia of severe infection may be unassociated with alteration of the bone marrow and the reason for the leukopenia is unexplained. On the other hand in patients with chronic neutropenia the bone marrow may reveal few myelocytes, promyelocytes and infrequent myeloblasts with an increase in reticular cells, lymphoid cells and plasma cells. In other cases a true maturation arrest occurs with striking hyperplasia of early myelocytes with a conspicuous absence of mature granulocytes.

**Causes** The causes of leukopenia may be summarized as follows:

- 1 Infections
  - A Bacterial
  - B Viral
  - C Miscellaneous
    - (1) Protozoal
    - (2) Rickettsial
  - D Overwhelming
- 2 Chemicals, drugs and physical agents
  - A Irradiation
  - B Therapeutic agents in systemic and blood disease
- 3 Miscellaneous
  - A With blood disorders
    - (1) Agranulocytosis
    - (2) Aplastic anemia and leukemia
    - (3) Hypoplastic anemia and leukopenia
    - (4) Periodic (cyclic) neutropenia
    - (5) Chronic hypoplastic neutropenia
    - (6) Chronic benign granulocytopenia in childhood
    - (7) Infantile genetic agranulocytosis
    - (8) Neonatal agranulocytosis
  - B Involving the spleen
    - (1) Primary and secondary splenic neutropenia
  - C Nutritional deficiencies

**PERIODIC (CYCLIC) NEUTROPENIA** Periodic neutropenia is a rare condition characterized by extreme leukopenia due to the disappearance of neutrophilic granulocytes from the circulating blood at approximately three week intervals and occurring at any age period but frequently beginning in infancy and childhood. The agranulocytic periods last ten days and are associated with the appearance of ulcers in the oral mucous membranes fever and sore throat Splenomegaly adenopathy arthralgia, abdominal pain, ischiorectal infections lymphadenitis, and conjunctivitis have also been reported.<sup>2-4</sup> As in patients with other kinds of leukopenia, when the white count is lowest mouth ulcerations gingivitis and often attacks of furunculosis are prone to appear.

In general the fluctuations of the circulating neutrophils are a reflection of the cyclic changes in the bone marrow. The granulocytes and their precursors may disappear from the bone marrow before the onset of neutropenia and reappear just prior to the return of neutrophils in the peripheral blood.<sup>2</sup> Often the bone marrow is depleted of mature forms during the agranulocytic stage but an abundance of early granulocytic forms constituting a "maturation arrest" is found.

Recurrent neutropenia has been described<sup>1</sup> in a patient with onset of disease in infancy marked by furunculosis, aphthous stomatitis and fever who was followed until death as a young adult. The three week cycle of neutropenia persisted with the leukocytes during these episodes numbering 2,000 to 4,000 per cubic millimeter and the granulocytes 6 to 10 per cent.

The pathogenesis of these syndromes the reason for their periodicity and the tendency for ulceration of the buccal mucosa have not been explained. No definite relationship to hormonal imbalance or the menstrual cycle has been established. It is not yet clear whether antileukocytic antibodies play a role in causation.

**CHRONIC HYPOPLASTIC NEUTROPENIA** In chronic hypoplastic neutropenia the course is extremely chronic and marked by repeated infections involving the skin and oral cavity and frequently by symptom free intervals. Splenomegaly of slight to moderate degree is present in all patients. The marked hypoplasia of granulocytic precursors of the bone marrow and the failure to respond to splenectomy differentiates this condition from primary splenic neutropenia in which granulocytic hyperplasia is present in the marrow and benefit is obtained by splenectomy.

**CHRONIC BENIGN GRANULOCYTOPENIA IN CHILDHOOD** Chronic benign granulocytopenia with leukopenia has been reported frequently. The bone marrow in these patients is usually normal but in some there is a disturbance in maturation of myelocytes to the segmented forms. Spontaneous remissions with eventual recovery may occur in a period of months to years.<sup>21</sup> In some children there is a lowered resistance to infection whereas in others neutropenia with leukopenia which persists for years without causing ill health but with a transient rise of granulocytes with severe infection is discovered as a chance finding. In one such child the granulocytes numbered below 10 per cent, with white blood counts ranging from those observed in leukopenia to normal counts. This persisted from the ages of 2 to 7 years with a complete and lasting remission following tonsillectomy. Rarely chronic neutropenia occurs in several siblings of a family.

The bone marrow usually shows a reduction in the total count of nucleated cells and a depression of the myeloid elements leaving the red cell precursors and megakaryocytes relatively undisturbed. The pathologic changes in the bone marrow are extremely variable and depend upon the stage of the disease when bone marrow is aspirated. Following injury there is a gradual disappearance of the myeloblasts, promyelocytes, myelocytes, and metamyelocytes and eventually of the more mature neutrophilic cells. These elements are replaced in the bone marrow by plasma cells, reticuloendothelial elements, and lymphocytes. Occasionally in this phase a proliferation of myeloblasts occurs. During recovery and in the patient with the mild form that has not progressed, the less mature myeloid forms reappear and are followed by the segmented neutrophilic cells.

Repeated bone marrow studies are required, however, to guard against a sudden shift to an immature level. In patients with mild injury both promyelocytes and myelocytes are present and a marked reduction in the more mature elements and an increase in stem cells and lymphocytes require immediate withdrawal of the offending drug. The prognosis therefore is favorable if normal numbers of granulocytes are observed in the marrow and is unsatisfactory if only lymphocytes and plasma cells are present.

Since the bone marrow returns to normal before mature granulocytes are liberated in the peripheral blood, prognosis depends upon the cellular composition of the marrow. Except for antimicrobial agents and antibiotics the effectiveness of the drugs usually prescribed for treatment of agranulocytosis must be correlated with the myelopoietic content of the marrow.

When penicillin or other antibiotics administered to combat infection are found to cause agranulocytosis, recovery from the agranulocytosis following their withdrawal probably is spontaneous and basically depends upon the ability of intrinsic factors supplied by the patient to stimulate granulopoiesis.

**LEUKOPENIA IN APLASTIC ANEMIA AND LEUKEMIA.** In children a persistent neutropenic leukopenia with or without purpura frequently signifies the presence of aplastic anemia or leukemia. One of the commonest features of childhood leukemia is the occurrence of leukopenia with predominance of lymphocytes and a moderate anemia during the course of an unexplained low grade fever associated with bouts of markedly elevated temperatures. Examination of the peripheral blood normally reveals a sparsity of platelets and occasionally immature white blood cells, whereas bone marrow examination usually demonstrates heavy infiltration with leukemic cells.

**HYPOPLASTIC ANEMIA AND LEUKOPENIA.** Leukopenia may occur in patients with mild hypoplastic anemia. In one instance fatal idiopathic aplastic anemia occurred in first cousins and a mild anemia and protracted leukopenia which extended from childhood into adult life was observed in a sister of one of them. In addition to oral ulceration, susceptibility to furunculosis and skin infiltration is common with intervals of good health between episodes despite the persistent leukopenia. The bone marrow in these patients shows a greater proportion of myeloid cells of all grades of maturity. A similar bone marrow picture was observed in a child with persistent leukopenia and neutropenia in whom febrile episodes occurred when the leukopenia was most marked.



**PERIODIC (CYCLIC) NEUTROPHENIA** Periodic neutropenia is a rare condition characterized by extreme leukopenia due to the disappearance of neutrophilic granulocytes from the circulating blood at approximately three week intervals and occurring at any age period but frequently beginning in infancy and childhood. The agranulocytic periods last ten days and are associated with the appearance of ulcers in the oral mucous membranes, fever and sore throat. Splenomegaly, adenopathy, arthralgia, abdominal pain, ischiorectal infections, lymphadenitis and conjunctivitis have also been reported.<sup>1</sup> As in patients with other kinds of leukopenia, when the white count is lowest, mouth ulcerations, gingivitis and often attacks of furunculosis are prone to appear.

In general, the fluctuations of the circulating neutrophils are a reflection of the cyclic changes in the bone marrow: "The granulocytes and their precursors may disappear from the bone marrow before the onset of neutropenia and reappear just prior to the return of neutrophils in the peripheral blood." Often the bone marrow is depleted of mature forms during the agranulocytic stage but an abundance of early granulocytic forms constituting a "maturation arrest" is found.

Recurrent neutropenia has been described<sup>1</sup> in a patient with onset of disease in infancy marked by furunculosis, aphthous stomatitis and fever who was followed until death as a young adult. The three week cycle of neutropenia persisted with the leukocytes during these episodes numbering 2,000 to 4,000 per cubic millimeter and the granulocytes 6 to 10 per cent.

The pathogenesis of these syndromes, the reason for their periodicity and the tendency for ulceration of the buccal mucosa have not been explained. No definite relationship to hormonal imbalance or the menstrual cycle has been established. It is not yet clear whether antileukocytic antibodies play a role in causation.

**CHRONIC HYPOPLASTIC NEUTROPENIA** In chronic hypoplastic neutropenia<sup>7</sup> the course is extremely chronic and marked by repeated infections involving the skin and oral cavity and frequently by symptom free intervals. Splenomegaly of slight to moderate degree is present in all patients. The marked hypoplasia of granulocytic precursors of the bone marrow and the failure to respond to splenectomy differentiates this condition from primary splenic neutropenia in which granulocytic hyperplasia is present in the marrow and benefit is obtained by splenectomy.

**CHRONIC BENIGN GRANULOCYTOPENIA IN CHILDHOOD** Chronic benign granulocytopenia with leukopenia has been reported frequently. The bone marrow in these patients is usually normal but in some there is a disturbance in maturation of myelocytes to the segmented forms.<sup>7</sup> Spontaneous remissions with eventual recovery may occur in a period of months to years.<sup>21</sup> In some children there is a lowered resistance to infection whereas in others neutropenia with leukopenia which persists for years without causing ill health but with a transient rise of granulocytes with severe infection is discovered as a chance finding. In one such child the granulocytes numbered below 10 per cent with white blood counts ranging from those observed in leukopenia to normal counts. This persisted from the ages of 2 to 7 years with a complete and lasting remission following tonsillectomy. Rarely, chronic neutropenia occurs in several siblings of a family.

ranging in severity from the mild to fatal case. Continuous treatment with antibiotics has been recommended as a means of rendering the child with chronic neutropenia free from infections.<sup>16</sup>

A form of this disease has been reported in adults whose course is marked by attacks of severe infection from relatively trivial causes. Cutaneous sepsis and inflammation of the gums, lips, mouth and throat are common with free intervals of comparative good health.<sup>1</sup> The bone marrow varies from hypoplasia to hyperplasia.

**INFANTILE GENETIC AGRANULOCYTOSIS** A variant of chronic benign granulocytopenia in childhood is infantile genetic agranulocytosis in which a severe depletion of granulocytes frequently with leukopenia is transmitted on the basis of simple recessive inheritance.<sup>11</sup>

**NEONATAL AGRANULOCYTOSIS** Lubhy and Slobody<sup>14</sup> observed transient agranulocytosis in successive siblings in the neonatal period. Such a circumstance was explained by transplacental isoimmunization of the mother to a leukocyte factor of her infant in a manner analogous to Rh isoimmunization causing hemolytic disease of the newborn infant.<sup>15</sup> In these patients agranulocytosis persisted for three to four weeks and in one of the infants it was accompanied by pulmonary infection. The predominance of neutrophilic myelocytes in the bone marrow may represent either a maturation arrest or a depletion of mature cells because of their increased agglutination and destruction in the peripheral blood.

Infants born to mothers with chronic neutropenia have revealed a transitory neutropenia which presumably was caused by the transplacental passage of a neutropenic factor.<sup>8</sup> In one case the mother's serum contained a demonstrable leukoagglutinin which could be transferred by a transfusion of plasma to a normal donor with a resulting severe neutropenia. The neonatal neutropenia persisted for three weeks. Hyperplasia of the granulocytic series with a maturation arrest at the myelocytic stage was present in the bone marrow of the affected infants.

Multiple cases of neonatal neutropenia occurring in one family were traced to a potent leukoagglutinin found in the maternal serum which agglutinated the leukocytes obtained from the father and three available children.<sup>11a</sup> On the other hand leukocyte agglutinins were absent in a patient with severe granulocytopenia associated with infection from 1 week of age which terminated fatally at 13½ months of age.<sup>8</sup> The serum electrophoretic pattern was normal. The bone marrow revealed an arrest at the myelocytic stage.

**SPLENIC NEUTROPENIA** Primary splenic neutropenia<sup>2</sup> is characterized by profound leukopenia and neutropenia, fever, ulcerations of the mucous membranes, susceptibility to infection, splenomegaly and myeloid hyperplasia of the bone marrow in which there may be an associated depression of the red cells and platelets. This has been regarded as a form of hypersplenism in which the leukocytes are involved in splenic hyperactivity. Relief of all symptoms results from splenectomy. In patients with secondary splenic neutropenia leukopenia occurs with disorders involving the spleen such as Banti's disease (portal hypertension), Gaucher's disease, lymphosarcoma and Hodgkin's disease. It is not yet certain whether the leukopenia in the primary or secondary syndromes results from

hypersequestration and hyperdestruction of granulocytes by and within the spleen or from inhibition by the spleen through a hormonal effect or by both mechanisms

**ALYMPHOCTOSIS** Alymphocytosis is a rare fatal disease occurring in infants and young children<sup>3</sup> which is characterized by fever hepatosplenomegaly and leukopenia with an absolute reduction of all types of leukocytes especially the lymphocytes Pancytopenia occurs terminally Postmortem examination reveals a widespread and generalized atrophy and necrosis of lymphoid tissue Infection with *Candida albicans* (moniliasis) was a common factor in patients reported on From their pathologic findings Janeway and Gitlin<sup>9</sup> suspect the presence of agammaglobulinemia

**NUTRITIONAL DEFICIENCIES** In experimental animals nutritional deficiencies induce leukopenia and granulocytopenia Leukopenia granulocytopenia, and occasionally anemia develop in rats fed a purified diet deficient in riboflavin<sup>4</sup> Folic acid corrects leukopenia and granulocytopenia can be corrected by the administration of protein<sup>10</sup> In patients in whom white blood cell regeneration was promoted by dietary protein granulocytes were found to react to a greater degree than lymphocytes and monocytes<sup>6</sup>

**Treatment** In deciding on treatment it must be remembered that many diseases such as leukemia aplastic anemia disseminated lupus erythematosus and lymphosarcoma may be preceded by a period of prolonged and undiagnosed leukopenia One child with neutropenia and repeated respiratory infections who was followed from 3 to 6 years of age eventually succumbed with a pathologic diagnosis of lymphosarcoma In this patient splenectomy was unsuccessful in altering the blood picture

The first steps in the treatment of neutropenia should aim toward the elimination of suspected drugs or chemicals and removal of the patient to an environment free from possible exposure to offending agents In the event of recurrent infections antibiotics should be prescribed to offset the effect of granulocytopenia with the hope that myeloid tissue will regenerate spontaneously Mouth ulcerations require urgent local treatment

Etiologic agents are usually difficult to ascertain and the problem of therapy is one of stimulating granulocytic proliferation Unfortunately such specific agents are not yet available and treatment on the whole is unsatisfactory Definitive treatment consists of prophylactic and therapeutic administration of antibiotics and the adrenocortical hormones and splenectomy None of these measures significantly alters the blood picture of patients with the cyclic or other neutropenias although ACTH the steroid hormones and splenectomy produce some degree of symptomatic relief especially with respect to fever and oral ulcers Pentnucleotide liver extract, vitamins and bone marrow extracts and leukocytic products have been ineffective in reversing leukopenia and granulocytopenia

Permanent and spontaneous remissions have been observed after prolonged periods of leukopenia especially when recurrent infections have been mild No clear-cut distinction can always be made between clinical syndromes that have in common leukopenia and granulocytopenia Attempts are usually made to

explain the leukopenia on the basis of splenic hyperactivity. Only in patients with primary splenic neutropenia when all criteria are fulfilled does removal of the spleen lead to complete recovery.<sup>3</sup> In other patients in whom the bone marrow shows a myelocytic hyperplasia without splenic enlargement removal of the spleen only rarely results in improvement without altering the basic condition.<sup>4</sup>

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## *Leukocytosis, Leukemoid Reactions, and Lymphocytosis*

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### LEUKOCYTOSIS

Leukocytosis is the term used to designate an increase in the total number of white blood cells. Although such increase may be due to a preponderance of any one of the cellular elements, most often the term leukocytosis is used simply to imply an increase due to the neutrophils. Neutrophilia is the preferable term and differentiates this form of leukocytosis from lymphocytosis, monocytosis, and eosinophilia.

#### *Physiologic Leukocytosis*

Leukocytosis is physiologic when it occurs without associated infection or other demonstrable pathologic lesions. The high leukocyte count of the newborn infant, with maximal values of 38 000 per cubic millimeter on the first day of life, is well known. The great majority of babies at this time have an average of 10 000 to 25 000 leukocytes per cubic millimeter, although much lower counts have been noted. The leukocytosis is initially due to a preponderance of polymorphonuclear neutrophils, mainly with a single lobed or double lobed nucleus. Trilobed forms appear about the ninth day to the second week.<sup>1</sup> Wide variations are found both between different infants and between different counts in the same baby. A pronounced drop at the end of the third or fourth day, with white blood cells averaging 12 000 per cubic millimeter, is maintained until the end of the first year. After the end of the second week, lymphocytes predominate for the remainder of infancy.

Strenuous exercise, convulsive seizures, emotional disorders, fear, and agitation, and pregnancy and labor have been associated with elevated leukocyte counts. Whether or not leukocytosis is attributable to digestion has not been demonstrated conclusively.<sup>4</sup>

#### *Pathologic Leukocytosis*

The extent of leukocytosis or leukopenia in patients with infection has been related to specific factors present in inflammatory exudates.<sup>16</sup> According to this

concept the leukocyte level in the blood stream of patients with inflammatory conditions is the resultant of leukocytosis promoting and leukopenic factors respectively

Usually a neutrophilic leukocytosis with a shift to the left of the polymorphonuclear cells eosinopenia and lymphopenia point to infection. The reappearance of eosinophils and segmented neutrophilic leukocytes and the appearance of monocytes indicate the recovery phase of an illness<sup>14</sup>

Acute infections caused by cocci (staphylococci streptococci pneumococci etc.) are the most common causes of a neutrophilic increase. Because of the tendency of the leukocytes to form pus especially in localized areas examination of the white blood cell count of patients with inflammatory conditions serves as a valuable clinical and diagnostic procedure.

Neutrophilic leukocytosis occurs in patients with pneumonia scarlet fever rheumatic fever diphtheria diabetic coma azotemia poisoning by chemicals and drugs such as lead mercury and camphor acute hemorrhage malignant neoplasms with metastases to the bone marrow marked tissue destruction such as burns and sudden hemolysis of red cells. In patients with dehydration the extent of leukocytosis is gauged by comparison with elevation of the hemoglobin level and red cell count.

Because of the great lability of the hematopoietic system in early life the white blood cell response in younger patients may be disproportionate to the intensity of stimulation. Marked leukocytosis and an outpouring of immature cells into the peripheral blood may result from a moderate infection. In general excessively high white blood cell counts or leukopenia associated with a shift to the left of the polymorphonuclear cells and toxic granulation indicates a serious outlook, especially if accompanied by excessive numbers of immature white cells and an absence of eosinophils.

## LEUKEMOID REACTIONS

Leukocytosis refers to white blood cell counts above the normal high of 11 000 per cubic millimeter and leukopenia refers to counts under 4 000 per cubic millimeter. Leukemoid reactions are unusual blood responses<sup>15</sup> associated with exaggerated leukocytic reactions (approximately 50 000 per cubic millimeter and above) or the presence of immature white cells suggesting leukemia regardless of the leukocyte level without postmortem evidence of leukemia. Depending upon the cell type involved leukemoid reactions may be classified as granulocytic (myeloid) lymphocytic and monocytic. Most commonly especially in younger patients a leukemoid condition implies a hyperleukocytosis with a marked shift to immature neutrophilic granulocytes myelocytes metamyelocytes occasional promyelocytes and myeloblasts. This response in infants is frequently associated with normoblasts in the peripheral blood. It is differentiated from leukemia by the absence of anemia thrombocytopenia hemorrhagic phenomena, splenomegaly and lymphadenopathy and by the infrequent appearance of blasts.<sup>8</sup> Exceptionally many of these features may be present in leukemoid reactions.<sup>16</sup> The clinical course the transient period of the reaction without recurrence bone marrow aspiration and the absence of tissue infiltration

usually rule out leukemia. The leukemoid process also lacks the characteristic gap or hiatus leukemicus between the blast forms and polymorphonuclear neutrophils with few or no transition forms such as myelocytes.

Another method of differentiation is by the measurement of alkaline phosphatase activity by histochemical and biochemical methods. A high cellular activity of this enzyme is found in the neutrophilic granulocytes of normal persons and of those with leukemoid reactions due to pyogenic infection and negligible amounts are found in the leukocytes of those with chronic myelogenous leukemia.<sup>11-14</sup> This enzymatic activity is also found to be consistently very low in paroxysmal nocturnal hemoglobinuria. Low values are also found in patients with a variety of hematologic and nonhematologic diseases such as idiopathic thrombocytopenic purpura, myeloid metaplasia, infectious mononucleosis, pernicious anemia in relapse, collagen diseases and refractory or aplastic anemia.<sup>15</sup>

Hyperleukocytosis of the myeloid type occurs following pyogenic infection, metastatic carcinoma of bone, ulcerative colitis, severe hemorrhage, primary tuberculous infection in childhood and acute tuberculosis at any age period and in episodes of acute hemolysis in the course of an established chronic hemolytic anemia or following infection or drug therapy. Congenital leukemia may be difficult to differentiate from the leukemoid reaction occurring with sepsis in the newborn infant. A positive diagnosis of leukemia often cannot be made from the blood and bone marrow findings alone and evidence of organ infiltration with white cell precursors is necessary.

A leukemoid reaction with a predominance of lymphocytes occurs in patients with whooping cough, chickenpox and acute infectious lymphocytosis. In all of the patients with these conditions the majority of lymphocytes are normal with small numbers of younger forms with deep blue cytoplasm. In patients with infectious mononucleosis the increase in total leukocytes is seldom more than 20,000 per cubic millimeter. Elevations above this number are exceptional but the presence of diagnostic atypical lymphocytes readily separates this disease from leukemia.

Blood pictures similar to those of patients with myelocytic and lymphocytic leukemia<sup>16</sup> have been reported in patients with military tuberculosis. A leukemoid reaction with monocytosis may occur in patients with tuberculosis in childhood with a hematogenous spread. A count of 82,000 white blood cells per cubic millimeter with 44 per cent monocytes has been reported in a 16-year-old boy with generalized tuberculous adenitis.<sup>17</sup>

## LYMPHOCYTOSIS

There are few conditions in which a significant increase in lymphocytes occurs so that they are responsible for a moderate or marked leukocytosis. An absolute lymphocytosis is found in patients with acute and chronic (nonspecific) infectious lymphocytosis, infectious mononucleosis and pertussis and to a lesser extent in those with syphilis, tuberculosis and hyperthyroidism. A relative increase in lymphocytes is common in patients with conditions with a decreased number of granulocytes. A relative lymphocytosis is associated with the leukopenia of measles, German measles, exanthema subitum and brucellosis. It will



be recalled that following the first week of life lymphocytes normally predominate until the fourth or fifth year of life and a lymphocytosis occurring in this period must be evaluated from this standpoint. Occasionally in infancy a marked lymphocytosis transiently accompanies an infectious or postinfectious state which cannot be assigned to a specific cause.

### *Acute Infectious Lymphocytosis*

**Definition** Acute infectious lymphocytosis—<sup>3</sup> a specific disease entity of unknown etiology which is both infectious and contagious. It may occur sporadically or in epidemic form. The incubation period has been estimated to be between twelve and twenty one days. It is characterized by a hyperleukocytosis due to an increase in small mature lymphocytes in which the elevated blood levels persist for approximately two to seven weeks. It is a benign infection distinct from infectious mononucleosis, acute lymphoblastic and chronic lymphocytic leukemia and miscellaneous infections associated with a lymphocytosis. The clinical signs and symptoms may be so mild as to escape attention or the onset may be marked by varying degrees of constitutional reaction. A noteworthy feature is the absence of lymphadenopathy and enlargement of the spleen.

**Age** The highest incidence is in children from 1 to 14 years of age with most of the recorded cases occurring during the first ten years of life. Several cases have been reported in young adults.<sup>4</sup> In general the height of the leukocyte count tends to vary inversely with the age of the patient.

**Etiology** Attempts to identify a causative agent have thus far met with inconclusive results. Organisms obtained from routine nasopharyngeal cultures probably represent normal inhabitants or secondary invaders. They do not differ from the flora of patients exhibiting the usual hematologic response with similar respiratory infections. A bacterial or viral etiology has been postulated<sup>13</sup> but not confirmed.

**Epidemiology** The disease occurs sporadically as multiple cases in families and in institutional epidemics.<sup>1, 11, 12, 14</sup> It is therefore contagious but of a low degree of infectivity. It has been reported in North Central and South America, Europe and Africa. In the epidemic reports the majority of patients were devoid of positive physical findings. According to some reports the patients presented themselves with signs of respiratory infection or gastrointestinal distress principally diarrhea.<sup>15</sup> In the epidemics the peak white blood cell counts in the individual patients ranged from 15,100 to 147,000 per cubic millimeter and the lymphocytes ranged from 63 to 97 per cent.

**Pathology** Microscopic examination of lymph nodes in the few patients studied<sup>3</sup> revealed the striking proliferation of the lining reticuloendothelium with almost complete blockage of the sinuses by masses of these cells and degeneration of the lymph follicles.

**Clinical Features** The condition at times may be so mild as to escape attention or the onset may be marked in individual patients by varying degrees of constitutional reaction. Fever, upper respiratory infections, skin rashes, abdominal complaints and meningoencephalitic manifestations may be present. Accidental discovery as the result of routine blood examinations is not uncommon. par

ticularly in institutional epidemics. No consistent correlation is evident between the degree of leukocytosis and the severity of symptoms.

**Nasopharynx** A feature common to most of the patients in whom any manifestations were present is mild infection of the upper respiratory tract. The throat may be deeply injected at the time of the initial examination and a history of recent infection of the upper respiratory tract frequently is elicited.

**Nervous System** Increasing experience has shown that acute infectious lymphocytosis should be considered in the diagnosis of an acute febrile illness in which there are symptoms suggestive of central nervous system involvement. Headache, irritability, vertigo, and pain and slight stiffness of the back of the neck may occur without other signs of meningitis. The spinal fluid may show a slight pleocytosis with variations in the type of predominating cell, although a slight increase in lymphocytes has been reported when the spinal fluid count was positive. This disease may simulate poliomyelitis<sup>14</sup> since headache, stiffness of the neck, restlessness, fever, malaise, vomiting, sore throat, and identical spinal fluid changes may occur at the onset of both conditions. The blood counts readily separate both diseases. Differentiation between these conditions assumes particular importance when the symptoms occur during the summer and early fall.

**Skin** A generalized morbilliform eruption and less commonly a herpetic eruption have been observed in patients during the first week of the disease.

**Abdomen** Signs may be sufficiently pronounced to suggest an acute surgical condition with an elevated temperature, vomiting, and severe abdominal pain.<sup>3</sup> Diarrhea was the most prominent symptom in sixteen of twenty-eight patients in one epidemic.<sup>15</sup>

**Spleen and Lymph Nodes** An important diagnostic criterion of infectious lymphocytosis is absence of significant enlargement of the spleen and lymph nodes. Pre-existing cervical nodes, especially of the posterior chain, are usually sequelae of previous attacks of nasopharyngitis and are not directly related to the development of acute infectious lymphocytosis. Lymphadenopathy and enlargement of the spleen are absent unless they existed prior to the onset of the disease.

**Incubation Period** Because the abnormal white cell counts are unexpectedly encountered in the course of routine blood examinations, the exact onset and duration of the leukocytic changes are unknown. It is therefore difficult to designate a precise incubation period. From the available cases thus far reported, it has been possible to fix the approximate length of the incubation period between twelve and twenty-one days.

**Laboratory Findings** Highly significant features of diagnostic import are observed among the laboratory findings.

**Blood** The red cells, hemoglobin content, platelets, and sedimentation rate are normal if the disease is uncomplicated. When the constitutional reaction is severe, the hemoglobin content and the red blood cell count may drop to the lower limit of normal. The absence of anemia is an important diagnostic feature in differentiating this ailment from acute lymphoblastic and chronic lymphatic

leukemia and from the lymphocytosis occurring in prolonged postinfectious states. No deviations in bleeding or clotting time have been observed.

The outstanding feature in patients with acute infectious lymphocytosis



Fig. 32 Typical blood smear from a patient with acute infectious lymphocytosis ( $\times 400$ )



Fig. 33 Higher magnification of Fig. 32 ( $\times 1000$ ) showing that lymphocytes are mainly of the small variety and of uniform size and structure. Only occasionally are slightly larger or intermediate types encountered but these are also mature. (From Smith C. H. Acute Infectious Lymphocytosis: a Specific Infection. *JAMA* 123: 342, 1944.)

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is the hyperleukocytosis with the relative and absolute increase in normal small mature lymphocytes. White blood cell counts at the height of the disease are usually over 40 000 to 50 000 per cubic millimeter with a maximum recorded level over 100 000 per cubic millimeter. In the reported institutional epidemics the peak white blood cell counts in the individual patients ranged from 15 100 to 147 000 per cubic millimeter and lymphocytes from 60 to 97 per cent.<sup>1</sup> The lymphocytes are mainly the small variety and are of uniform size and normal structure. Only occasionally are slightly larger or intermediate types encountered but these are also mature cells. The nucleus in these cells is condensed and possesses the coarse chromatin masses of the normal mature lymphocyte. These lymphocytes have also been referred to as overripe and have been described as being smaller than normal with a dark purple chromatic material in the nucleus and with very little cytoplasm.<sup>17</sup>

The duration of the markedly abnormal leukocytic reaction is usually three to five weeks and occasionally seven weeks. An elevation in the percentage of eosinophilic leukocytes occurs frequently during the course of the disease usually at or following the peak of leukocytosis.

**Bone Marrow.** The myeloid elements and nucleated red cells are normal in number. In many patients the bone marrow shows an increase in the total number of nucleated cells and in the percentage of small mature lymphocytes otherwise it is not abnormal.

**Heterophil Agglutination (Paul Bunnell) Test.** The heterophil agglutination test is uniformly negative.

**Differential Diagnosis** (Table 15). Lymphocytic reactions in childhood are difficult to interpret because a predominance of lymphocytes and a greater lability of the blood forming mechanism are common to this age period. In the differentiation of acute infectious lymphocytosis these physiologic hematopoietic responses as well as certain specific conditions in which the lymphocytes or their precursors are known to be increased must be evaluated.

**Infectious Mononucleosis.** Infectious mononucleosis is usually more severe and characterized by fever, sore throat, rash, rarely jaundice, enlargement and tenderness of the lymph nodes and often splenomegaly. The febrile phase lasts for one to three weeks but enlargement of the glands and spleen may persist. Acute infectious lymphocytosis on the other hand may run an asymptomatic course or there may be transient sore throat, fever and constitutional symptoms. Lymph nodes and spleen are not enlarged. The heterophil antibody reaction is usually positive in patients with infectious mononucleosis but is consistently negative in those with acute infectious lymphocytosis.

The blood picture constitutes the most important differential feature. In patients with infectious mononucleosis the total white blood cell count usually does not exceed 20 000 per cubic millimeter whereas acute infectious lymphocytosis is characterized by a hyperleukocytosis with maximal levels frequently exceeding 50 000 cells per cubic millimeter. A more important feature of a hematologic differentiation rests in the morphologic appearance of the lymphocytes in the two conditions. In patients with acute infectious lymphocytosis the hyperleukocytosis is associated with a preponderance of small lymphocytes

Table 15 Differential Diagnosis of Acute Infections Lymphocytosis\*

|                              | Acute Infections<br>Lymphocytosis | Infectious<br>Mononucleosis   | Acute Lymphoblastic<br>(Stem Cell) Leukemia | Chronic Lymphocytic<br>Leukemia |
|------------------------------|-----------------------------------|-------------------------------|---------------------------------------------|---------------------------------|
| Age (usual incidence)        | First decade                      | First 3 decades               | First and second decades                    | After 15 years                  |
| Fever and systemic symptoms  | Occasionally present              | Usually present               | Variably present                            | Frequent                        |
| Enlarged lymph nodes         | Absent                            | Frequent                      | Frequent                                    | Frequent                        |
| Splenomegaly                 | Absent                            | Frequent in 50%               | Frequent                                    | Frequently pronounced           |
| Leukocytosis                 | Extreme                           | Moderate                      | Leukopenia to marked                        | Frequent                        |
| Lymphocytosis                | Frequent                          | Frequent                      | Frequent                                    | Frequent                        |
| Typical lymphocytic drainage | Normal small                      | Atypical abnormal             | Lymphoblasts                                | Mature small                    |
| Anemia                       | Absent                            | Rare                          | Frequent                                    | Late in course                  |
| Chromocytopenia              | Absent                            | Rare                          | Frequent                                    | Frequent                        |
| Bone marrow lymphocytosis    | Incidental in normal small        | Occasionally atypical         | Lymphoblasts present                        | Lymphocytes predominant         |
| Heterophil agglutination     | Negative                          | Positive                      | Negative                                    | Negative                        |
| Prognosis                    | Uniformly favorable               | Favorable with few exceptions | Uniformly fatal                             | Uniformly fatal                 |

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is the hyperleukocytosis with the relative and absolute increase in normal small mature lymphocytes. White blood cell counts at the height of the disease are usually over 40 000 to 50 000 per cubic millimeter with a maximum recorded level over 100 000 per cubic millimeter. In the reported institutional epidemics the peak white blood cell counts in the individual patients ranged from 15 100 to 147 000 per cubic millimeter and lymphocytes from 60 to 97 per cent.<sup>1</sup> The lymphocytes are mainly the small variety and are of uniform size and normal structure. Only occasionally are slightly larger or intermediate types encountered but these are also mature cells. The nucleus in these cells is condensed and possesses the coarse chromatin masses of the normal mature lymphocyte. These lymphocytes have also been referred to as overripe and have been described as being smaller than normal with a dark purple chromatic material in the nucleus and with very little cytoplasm.<sup>11</sup>

The duration of the markedly abnormal leukocytic reaction is usually three to five weeks and occasionally seven weeks. An elevation in the percentage of eosinophilic leukocytes occurs frequently during the course of the disease usually at or following the peak of leukocytosis.

**Bone Marrow.** The myeloid elements and nucleated red cells are normal in number. In many patients the bone marrow shows an increase in the total number of nucleated cells and in the percentage of small mature lymphocytes otherwise it is not abnormal.

**Heterophil Agglutination (Paul Bunnell) Test.** The heterophil agglutination test is uniformly negative.

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The blood picture constitutes the most important differential feature. In patients with infectious mononucleosis the total white blood cell count usually does not exceed 20 000 per cubic millimeter whereas acute infectious lymphocytosis is characterized by a hyperleukocytosis with maximal levels frequently exceeding 50 000 cells per cubic millimeter. A more important feature of a hematologic differentiation rests in the morphologic appearance of the lymphocytes in the two conditions. In patients with acute infectious lymphocytosis the hyperleukocytosis is associated with a preponderance of small lymphocytes

possessing a normal cytologic appearance. In those with infectious mononucleosis however the distinctive feature is the presence of characteristic atypical mononuclear cells. The variability of atypical lymphocytes and monocytes in persons with this disease contrasts sharply with the uniform size and normal structure of the cells in those with acute infectious lymphocytosis.

**Acute Lymphoblastic (Stem Cell) Leukemia** The extreme leukocytosis and the predominance of lymphocyte in patients with acute infectious lymphocytosis have occasionally led to the erroneous diagnosis of acute lymphoblastic leukemia. Identification of the blast cell which is invariably found in a careful search of the blood smear of patients with leukemia is the distinguishing feature. The size of these cells varies from that of a small lymphocyte to twice that size. Lymphoblasts are usually round, large and uniform in size and the narrow zone of cytoplasm is basophilic and occasionally contains a few large vacuoles.

Even more distinctive is the nuclear pattern. In contrast with the masses of dense basichromatin found in the nucleus of the normal and large lymphocyte of the patient with acute infectious lymphocytosis, the nuclear chromatin of the lymphoblast stains lightly, is finely granular, stippled or sievelike and nucleoli are present. In patients with acute leukemia with a hyperleukocytosis of the magnitude found in those with acute infectious lymphocytosis, the blood exhibits not only large numbers of lymphoblasts but also a severe anemia and a decrease in platelets and the spleen and lymph nodes are enlarged.

Examination of the bone marrow also serves to differentiate the two diseases. In acute lymphatic leukemia in children, bone marrow aspiration usually shows complete replacement of the bone marrow by blast or stem cells. In children with acute infectious lymphocytosis, on the other hand, the myeloid and erythroblastic elements are present in normal proportions and the chief abnormality in many patients consists of an increased percentage of normal lymphocytes.

**Chronic Lymphocytic Leukemia** The blood picture of patients with acute infectious lymphocytosis resembles that of those with chronic lymphocytic leukemia in respect to the white blood cells. In patients with either of these diseases there is a hyperleukocytosis with a preponderance of small mature lymphocytes and the bone marrow shows increased percentages of normal small lymphocytes and is cellular. The age incidence of the two diseases, however, is very different since chronic lymphocytic leukemia is a disease of older persons. In patients with chronic lymphocytic leukemia, the spleen and lymph nodes are enlarged, anemia and thrombocytopenia eventually develop and the outcome is uniformly fatal, in contrast to the negative physical and hematologic findings and favorable prognosis in patients with acute infectious lymphocytosis.

**Leukemoid Reactions** The hyperleukocytosis of infectious lymphocytosis can not be regarded as a leukemoid reaction of the lymphoid type. The absence of immature and atypical cells in the blood and bone marrow precludes this designation. Moreover, the leukemoid reaction resulting from infection is transitory. The lymphocytic response in patients with acute infectious lymphocytosis is protracted. Pertussis as a cause of the extreme leukocytosis and lymphocytosis can be excluded by the absence of cough and characteristic clinical manifestations.

**Miscellaneous Infections** A number of specific infections are associated with



lymphocytosis Typhoid fever brucellosis and tuberculosis should be excluded by appropriate tests

Available data render invalid the view that the lymphocytosis represents a peculiar individual response the so called constitutional lymphatic reaction Patients with acute infectious lymphocytosis afflicted with antecedent concurrent, or subsequent illnesses such as acute otitis media or pneumonia exhibit a neutrophilic response

**Treatment and Prognosis** There is no specific therapy Treatment is symptomatic and similar to that in patients with other acute infections of the upper respiratory tract Because of the low infectivity of the condition the large number of asymptomatic cases and the brief span of constitutional reactions in the febrile cases no isolation seems necessary The prognosis in all cases has been uniformly excellent and no sequelae have been observed

### *Chronic Nonspecific Infectious Lymphocytosis (Low Grade Fever Syndrome)*

**Clinical Picture** The symptom complex of chronic nonspecific infectious lymphocytosis is characterized by a persistent slight to moderate leukocytosis and a preponderance of lymphocytes It is frequently encountered in pediatric practice and is distinct from acute infectious lymphocytosis Difficulties in diagnosis arise when an acute infection of the upper respiratory tract is followed for prolonged periods by a low grade fever with the temperature ranging from 99° F up to and usually not including 101° F In addition to the protracted fever the symptom complex includes anorexia pallor irritability fatigability and abdominal pain localized to the region of the umbilicus The fauces are injected the tonsils when present are usually greatly hypertrophied and postnasal discharge is frequent At times the superficial cervical lymph nodes are slightly enlarged The heart and lungs are normal there are no murmurs or other cardiac abnormalities In infants and young children the spleen may be palpable but this finding is inconstant especially in older children The age of incidence is usually from 6 months through 10 years especially in the period from 3 to 6 years Cases are less commonly observed between 3 and 6 months of age In these infants moderate elevations of temperature (103° to 104° F) frequently interrupt the usual course of low grade fever (temperature up to 101° F) Although these episodes are usually related to exacerbations of infection of the nasopharynx other causes of lymphocytosis must be eliminated

**Blood** The accompanying slight to moderate leukocytosis and lymphocytosis usually persist for periods of months and sometimes a year or more The total white blood cell counts usually range from 8 000 to 18 000 rarely reaching 20 000 per cubic millimeter with 60 to 80 per cent lymphocytes White blood cell counts reaching 25 000 per cubic millimeter are frequently observed in infants under 6 months of age The hemoglobin ranges between 10 and 11 gm per 100 ml and the platelets are normal in number Lymphocytosis in this group of patients with chronic disease resembles that which occurs in postinfectious states in infants and children Most of the lymphocytes are of the small mature type structurally similar to those in patients with acute infectious lymphocytosis Oc

occasionally cells of the larger variety are seen which are of normal shape and which possess a deeply basophilic cytoplasm and an eccentric nucleus which stains more intensely than that of the normal lymphocyte. The same depth of staining is observed in another type of lymphocyte which is about one and one half times the size of a red cell and whose nucleus is round oval or slightly indented. In infants the zone of cellular cytoplasm is often wider and the basophilia deeper than in the corresponding cells of blood in older children. These larger cells are not specific since they appear in increased numbers in the blood of young children during the active stage and convalescence of a large variety of infections and may occasionally be found in the blood of normal infants.

**Differential Diagnosis.** Chronic nonspecific infectious lymphocytosis is not contagious and does not appear in epidemic form in contrast to acute infectious lymphocytosis. Moreover the acute disease is usually asymptomatic the blood reaction is comparatively short and well defined hyperleukocytosis is marked and the disease is not characterized at any age period by either lymphadenopathy or splenomegaly.

Chronic nonspecific infectious lymphocytosis is often confused with infectious mononucleosis especially in young children acute lymphoblastic leukemia and acute rheumatic fever. Not only is the heterophil agglutinin test negative in the patient with chronic infectious lymphocytosis but the lymphocytes lack the irregularity of shape and the abundant frequently vacuolated, and foamy cytoplasm which are distinctive features of the cells of patients with infectious mononucleosis.

Acute lymphoblastic (stem cell) leukemia is differentiated by the presence of lymphoblasts hyperleukocytosis often leukopenia anemia (hemoglobin below 10 gm per 100 ml) and thrombocytopenia. Marked lymphadenopathy and splenomegaly are additional confirmatory features but may be absent in the patient with early disease. Examination of the bone marrow serves to differentiate the two diseases unequivocally although this procedure is rarely necessary.

Many of the patients are referred with a diagnosis of acute rheumatic fever because of a more or less continuous low grade fever a failure to gain weight or loss of weight fatigability and abdominal pain. From the hematologic standpoint acute rheumatic fever is usually associated with moderate leukocytosis with polymorphonuclear leukocytes as the predominant cell in contrast to chronic nonspecific infectious lymphocytosis in which lymphocytosis characterizes the blood smear. The sedimentation rate is markedly increased in patients with rheumatic fever and does not approach normal until some time after the fever and clinical signs have abated whereas in those with chronic lymphocytosis the sedimentation rate is normal or only slightly increased for short intervals. Nosebleeds the development of carditis pains in the arms legs and joints subcutaneous nodules and erythematous rashes are other features present in patients with rheumatic fever and absent in those with lymphocytic diseases.

A disease identical with or similar to chronic infectious lymphocytosis has been reported as lymphocytic fever.<sup>6</sup> It occurs in children chiefly under 2 years of age with enlargement of the liver and spleen slight lymphadenopathy

and a leukocytosis with a preponderance of small lymphocytes persisting for months and occasionally a year or more

The lymphocytic blood picture in patients with chronic infectious lymphocytosis is a useful diagnostic aid in differentiating the abdominal pain which may occur in both diseases from surgical conditions and from nonspecific mesenteric lymphadenitis which are associated with a neutrophilic response

**Treatment and Prognosis** Chronic nonspecific infectious lymphocytosis may be a source of anxiety to both parents and physician It is therefore desirable to emphasize that the prognosis is entirely favorable that treatment is symptomatic and that subsidence of the disease depends upon relief of infection in the nasopharynx Striking improvement often follows tonsillectomy but tonsillectomy is not advocated as a routine measure

Repeated temperature readings are unnecessary and antibiotics are futile in altering the pattern of low grade fever Once the diagnosis is established restriction of activities is unnecessary

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## Infectious Mononucleosis

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**Definition** Infectious mononucleosis is an acute infectious disease of unknown etiology. It is usually benign, has a self-limited course, and is characterized by lymphocytosis with atypical lymphocytes in the peripheral blood, enlarged lymph nodes and spleen, and various other constitutional symptoms. In a large number of patients the serum reveals a high titer of agglutinins for sheep red cells.

**Historical** The earliest descriptions of the disease were by Filatov<sup>11</sup> in 1885 and by Pfeiffer<sup>2</sup> in 1889 who gave it the name glandular fever. The latter reported a symptom complex in children marked by an acute onset and enlargement of the posterior cervical nodes, the liver, and spleen. Case reports of glandular fever appeared in the literature in increasing numbers, emphasizing the clinical aspects of the disease. In 1920 Sprunt and Evans<sup>11</sup> drew attention to its infective nature, described the changes in the peripheral blood characterized by an increase in mononuclear and other normal cells, and designated the disease as infectious mononucleosis. Soon afterward Longcope<sup>27</sup> and Downey and McKinlay<sup>6</sup> provided an accurate morphologic description of the cells diagnostic of this disease. The three types of cells described by the latter are frequently referred to as Downey cells. Tidy and Daniel at this time<sup>45</sup> confirmed the significance of these cells and pointed out that glandular fever and infectious mononucleosis were identical conditions. From that time until the serologic studies were made, this disease received increasing recognition, and both the clinical features and blood picture became more sharply defined.

A basic contribution to the diagnosis of infectious mononucleosis was made in 1932 with the discovery by Paul and Bunnell<sup>30</sup> that the serum of patients with this disease agglutinated the washed erythrocytes of sheep in concentrations above a normal titer. These agglutinins are termed heterophil antibodies because the reaction is with antigens of a different species that has nothing to do with their development—in this case, sheep cells. This discovery stimulated renewed interest in this disease, particularly in its immunologic aspects, and provided a diagnostic criterion of great accuracy in a disease with such protean manifestations.

**Pathology** The focal cellular and perivascular infiltration with normal and

abnormal lymphocytes in so many of the organs such as the liver nervous system kidneys heart and lungs accounts in large measure for the variety of clinical symptoms

Atypical lymphocytes are not present in the bone marrow although a shift to the left of polymorphonuclear neutrophils with increased numbers of myelocytes occasionally is observed In the lymph nodes the nodal architecture is maintained and marked proliferative activity of the pulp with picking by lymphocytes is present Throughout the pulp in the sinuses and on the edges of the germinal centers large numbers of the atypical mononuclear cells observed in patients with infectious mononucleosis have been described<sup>14</sup>

**Clinical Features** The problem of diagnosis usually is resolved by combining the heterophil antibody test with the hematologic and clinical findings especially since seronegative cases of this disease have occurred In early stages of the disease the diagnosis is puzzling since enlarged and tender lymph nodes characteristic blood abnormalities and a positive heterophil test often do not appear until the end of the first week or ten days

In recent years also the involvement of the liver and to a lesser extent the peripheral and central nervous systems has been reported with sufficient frequency to serve as an accessory diagnostic feature The causative agent has not yet been identified although a virus etiology is suspected<sup>9</sup> The disease appears in both epidemic and sporadic forms It is infectious but manifests a low degree of contagiousness It occurs at any age most commonly in children and young adults and rarely in infants The incubation period is given as four to fourteen days

The disease is marked by fever sore throat and epistaxis frequently with acute tonsillitis and often with generalized enlargement of the lymph nodes and spleen The edge of the spleen is soft and the organ is never greatly enlarged The spleen is palpable in approximately 50 per cent of patients Other clinical features to attract attention include headache vomiting urticaria rash jaundice periorbital edema and pain referable to the external muscles of the eyes Abdominal pain may be a predominant feature and the signs and symptoms may simulate acute appendicitis Appendectomies have been performed before the true nature of the condition was recognized<sup>43</sup> Hematuria and rectal bleeding have been encountered<sup>45</sup> but are rare in children

In a few instances an enanthem consisting of pinhead sized spots which darken in three to four days appears on the palate True petechiae numbering ten to several hundred clustered near the junction of soft and hard palate and more posteriorly close to the midline have also been described<sup>38</sup> They appear three days to ten weeks after the onset of symptoms and last three to eleven days These have not been associated with a hemorrhagic diathesis

An exanthem occurs more frequently The rash may be macular maculopapular purpuric or morbilliform resembling rubella Rose spots simulating those found in patients with typhoid fever have also been observed Falsely positive serologic reactions for syphilis<sup>44</sup> also have been encountered occasionally in the first week of the disease but revert to negative between the third and tenth weeks In association with a rubelliform rash and lymphadenopathy differential

tion of syphilis from infectious mononucleosis may be difficult in the early weeks

**Clinical Types** Although symptoms vary widely they have been classified by some authors into several clinical categories. The glandular or childhood type is marked by sore throat fever and generalized lymphadenopathy and frequently occurs in epidemics. In young adults the pharyngeal type is common and is marked by fatigue and sore throat general malaise low grade fever and slight to moderate enlargement of lymph nodes. In patients with the febrile type the onset is sudden often with prolonged typhoidlike fever headaches chills and prostration followed later by rash and lymph node and splenic enlargement. Less commonly the onset is ushered in variously as an acute allergic reaction with angioneurotic edema jaundice the signs of meningoencephalitis or a severe angina with a membrane on or near the tonsils which mimics diphtheria.

In some children the initial period of illness extending for five to seven days may be marked by the temperature reaching levels of hyperpyrexia with no other abnormal physical signs than a slightly palpable spleen. There may be no complaints. Leukocytes range from 5 000 to 6 000 per cubic millimeter with a predominance of polymorphonuclear neutrophils. After several days of fever a diffuse eruption resembling rubella may appear without the enlargement of the postauricular postcervical and occipital lymph nodes which marks this disease. At the end of a week temperatures are lower eruptions are fading and a moderate leukocytosis with a predominance of lymphocytes and a substantial number of diagnostic cells of infectious mononucleosis is noted. Throughout the entire extent of the disease symptoms and physical signs may be minimal.

In rare instances the early stages of infectious mononucleosis simulate leukemia both clinically and hematologically and careful scrutiny of blood smears and heterophil antibody tests is needed to differentiate between the two conditions. Of the clinical features that have been enumerated the presence of unexplained fever posterior cervical adenopathy pharyngitis and a tonsillar exudate should prompt a careful examination of the blood smear for the atypical cells found in patients with infectious mononucleosis.

**Involvement of the Nervous System** The pathologic changes in patients with infectious mononucleosis are widespread and include changes in the central nervous system in the liver and to a lesser extent in the heart and spleen. Involvement of the nervous system has been increasingly recognized in children as well as in adults. It has been stated<sup>1</sup> that this disease should be considered in the differential diagnosis of encephalitis and meningitis for which no specific cause has been found. The nervous system manifestations vary from intense headache blurred vision and stiffness of the neck to definite evidence of central nervous system involvement. Encephalitis encephalomyelitis serous meningitis acute polyradiculoneuritis as observed in patients with the Guillain Barre syndrome facial palsy retrobulbar neuritis and other signs of isolated or diffuse upper and lower motor neuron lesions have been described. Although the association of seizures with infectious mononucleosis is infrequent this disease should be considered in the differential diagnosis of unexplained and ill defined convulsive episodes.<sup>2</sup> Alterations in the spinal fluid may occur to the extent of a moderate pleocytosis increased pressure increase in mononuclear cells and less frequently

increased protein Neurologic complications appear two to four weeks after the onset of infectious mononucleosis but may precede or occur concomitantly with it Recovery from the neurologic complications is the rule although permanent damage and even death from respiratory paralysis have been noted <sup>8</sup>

*Involvement of the Liver* Increasing evidence supports the view that impaired liver function accompanies the majority of cases of this disease Jaundice may occur as the initial symptom or later in the disease with enlargement of lymph nodes More frequently hepatitis in the absence of jaundice has been reported in many studies as revealed with the use of serial liver function tests In an examination of sixty two children with a presumptive diagnosis of infectious mononucleosis Hsia and Gellis<sup>10</sup> have shown 84 per cent to have abnormal liver function It has been suggested therefore that tests for liver function such as cephalin flocculation thymol turbidity and Bromsulphalein retention tests be used to supplement other diagnostic tests for infectious mononucleosis Similarly in patients suspected of having infectious hepatitis it may be advisable to test for heterophil antibodies Patients especially children with infectious mononucleosis however recover quickly and completely whereas the course of those with infectious hepatitis is more prolonged and complicated The impaired liver function may also account for prolonged convalescence and for the easy exhaustion and weakness found especially in young adults though less commonly in children Pathologic examination of the liver reveals a varying picture—from a normal architecture to focal hepatitis with loss of liver cells cellular infiltration by atypical mononuclear cells dilatation and invasion of sinuses areas of scattered focal necrosis and enlarged portal lymph nodes The usual course of hepatitis is two to six weeks with a rapid return to normal <sup>8</sup>

*Involvement of the Heart* Cardiac complications occur infrequently especially in childhood and are detected by electrocardiographic and clinical examinations These have been attributed to focal interstitial infiltrations of mononuclear cells and lymphocytes in the myocardium <sup>1</sup> Fatal acute myocarditis with focal microscopic areas of necrosis and fragmentation of heart muscle fibers has been reported <sup>1</sup> Although scattered necropsy reports have shown involvement of the myocardium there is every evidence that valvular heart disease is a rare complication of infectious mononucleosis <sup>17</sup> The changes observed on the electrocardiogram are transient and consist of lowering or inversion of the T waves in various leads and increased P R interval Since clinical examination infrequently reveals an affected heart the correct interpretation of these electrocardiographic findings remains uncertain <sup>18</sup> The first symptoms of infectious mononucleosis may be those of acute benign pericarditis <sup>15 30</sup> Although patients with fatal nervous system involvement or splenic rupture are more likely to show definite myocarditis chronic heart disease is not to be expected as a sequela in patients with infectious mononucleosis

*Rupture of the Spleen* Rupture of an enlarged spleen can occur spontaneously or as a result of trauma and has also been related to vigorous palpation of the abdomen The liability to rupture is not encountered until the third week of illness and is due to the softness and friability of the spleen resulting from thinning of the capsule and dissolution of the trabeculae by lymphocytic infiltration <sup>4</sup>



**Laboratory Findings** Characteristic abnormalities are found in the peripheral blood and by serologic examination

**Blood Picture** In the majority of patients the blood picture constitutes the most important diagnostic feature of infectious mononucleosis. The total white blood cell count usually ranges from 10 000 to 20 000 per cubic millimeter during the peak of the disease in the second and third weeks and exceeds the upper limit to 50 000 in rare cases. At such extreme levels it must be differentiated from acute infectious lymphocytosis. Leukocytosis usually accompanies fever and enlargement of the lymph nodes. The count however varies greatly and leukopenia with white blood cell levels as low as 3 000 per cubic millimeter may mark the disease in its first week. Frequently in young adults the white blood cell count is normal but clinical symptoms and the blood smear provide the clue to diagnosis.

The striking feature in the blood smear is the shift from an early normal or increased percentage of polymorphonuclear neutrophils to a predominance of the mononuclear elements to the extent of 50 to 90 per cent. The term mononuclear cells was formerly applied to both monocytes and lymphocytes—hence the term infectious mononucleosis. The majority of abnormal cells are now known to be derivatives of the lymphocytic series and must be differentiated from the immature lymphocytes of leukemia. The atypical mononuclear cell in the blood of patients with infectious mononucleosis represents its distinctive feature. The identification of lymphocytes however has been verified by supravital staining. Monocytes may also be increased. The characteristic mononuclear cells consisting mainly of lymphocytes are larger than normal their cellular edges are often ragged and irregular and the cytoplasm is usually abundant staining a darker blue with Wright's stain than do normal large lymphocytes. The cytoplasm contains fine or coarse azure granules and one of the most striking features is the vacuolation. The vacuoles are of two types they may be large and scattered in cells with abundant cytoplasm or they may be smaller and more numerous. The combination of fine vacuolation and basophilic gives the cytoplasm a distinctive foamy appearance.

The nucleus of the cells in patients with infectious mononucleosis may be round oval or kidney shaped occasionally it is divided and often eccentrically placed. The Downey type I cell refers to the cells with dark blue foamy cytoplasm and an irregular kidney shaped nucleus. The Downey type II cell possesses larger amounts of light blue cytoplasm and an eccentrically placed nucleus of normal appearance. The diagnosis in the acute stages of infectious mononucleosis is made by the presence of one or another of the characteristic types of cells and by the assortment of unusual cells in the blood smear.

Occasionally small numbers of large rounded cells with well differentiated clear nuclei and lightly basophilic cytoplasm resembling reticuloendothelial cells appear in the peripheral blood. They probably originate from areas of reticulum cell proliferation in affected lymph nodes.<sup>10</sup>

Just as agglutination may appear in higher titer in patients with diseases other than infectious mononucleosis so have these atypical cells been noted in patients with a variety of conditions such as infectious hepatitis virus pneumonia German measles, measles acute brucellosis influenza roseola infantum and others.

increased protein. Neurologic complications appear two to four weeks after the onset of infectious mononucleosis but may precede or occur concomitantly with it. Recovery from the neurologic complications is the rule although permanent damage and even death from respiratory paralysis have been noted.<sup>8</sup>

*Involvement of the Liver* Increasing evidence supports the view that impaired liver function accompanies the majority of cases of this disease. Jaundice may occur as the initial symptom or later in the disease with enlargement of lymph nodes. More frequently hepatitis in the absence of jaundice has been reported in many studies as revealed with the use of serial liver function tests. In an examination of sixty-two children with a presumptive diagnosis of infectious mononucleosis Hsia and Gellis<sup>19</sup> have shown 84 per cent to have abnormal liver function. It has been suggested therefore that tests for liver function such as cephalin flocculation, thymol turbidity, and Bromsulphalein retention tests be used to supplement other diagnostic tests for infectious mononucleosis. Similarly, in patients suspected of having infectious hepatitis it may be advisable to test for heterophil antibodies. Patients, especially children with infectious mononucleosis, however, recover quickly and completely, whereas the course of those with infectious hepatitis is more prolonged and complicated. The impaired liver function may also account for prolonged convalescence and for the easy exhaustion and weakness found especially in young adults though less commonly in children. Pathologic examination of the liver reveals a varying picture—from a normal architecture to focal hepatitis with loss of liver cells, cellular infiltration by atypical mononuclear cells, dilatation and invasion of sinuses, areas of scattered focal necrosis, and enlarged portal lymph nodes. The usual course of hepatitis is two to six weeks with a rapid return to normal.<sup>9</sup>

*Involvement of the Heart* Cardiac complications occur infrequently, especially in childhood, and are detected by electrocardiographic and clinical examinations. These have been attributed to focal interstitial infiltrations of mononuclear cells and lymphocytes in the myocardium.<sup>1</sup> Fatal acute myocarditis with focal microscopic areas of necrosis and fragmentation of heart muscle fibers has been reported.<sup>1</sup> Although scattered necropsy reports have shown involvement of the myocardium, there is every evidence that valvular heart disease is a rare complication of infectious mononucleosis.<sup>17</sup> The changes observed on the electrocardiogram are transient and consist of lowering or inversion of the T waves in various leads and increased P-R interval. Since clinical examination infrequently reveals an affected heart, the correct interpretation of these electrocardiographic findings remains uncertain.<sup>18</sup> The first symptoms of infectious mononucleosis may be those of acute benign pericarditis.<sup>15, 20</sup> Although patients with fatal nervous system involvement or splenic rupture are more likely to show definite myocarditis, chronic heart disease is not to be expected as a sequela in patients with infectious mononucleosis.

*Rupture of the Spleen* Rupture of an enlarged spleen can occur spontaneously or as a result of trauma and has also been related to vigorous palpation of the abdomen. The liability to rupture is not encountered until the third week of illness and is due to the softness and friability of the spleen resulting from thinning of the capsule and dissolution of the trabeculae by lymphocytic infiltration.<sup>4</sup>

**Laboratory Findings** Characteristic abnormalities are found in the peripheral blood and by serologic examination

**Blood Picture** In the majority of patients the blood picture constitutes the most important diagnostic feature of infectious mononucleosis. The total white blood cell count usually ranges from 10 000 to 20 000 per cubic millimeter during the peak of the disease in the second and third weeks and exceeds the upper limit to 50 000 in rare cases. At such extreme levels it must be differentiated from acute infectious lymphocytosis. Leukocytosis usually accompanies fever and enlargement of the lymph nodes. The count however varies greatly and leukopenia with white blood cell levels as low as 3 000 per cubic millimeter may mark the disease in its first week. Frequently in young adults the white blood cell count is normal but clinical symptoms and the blood smear provide the clue to diagnosis.

The striking feature in the blood smear is the shift from an early normal or increased percentage of polymorphonuclear neutrophils to a predominance of the mononuclear elements to the extent of 50 to 90 per cent. The term mononuclear cells was formerly applied to both monocytes and lymphocytes—hence the term infectious mononucleosis. The majority of abnormal cells are now known to be derivatives of the lymphocytic series and must be differentiated from the immature lymphocytes of leukemia. The atypical mononuclear cell in the blood of patients with infectious mononucleosis represents its distinctive feature. The identification of lymphocytes however has been verified by supravital staining. Monocytes may also be increased. The characteristic mononuclear cells consisting mainly of lymphocytes are larger than normal their cellular edges are often ragged and irregular and the cytoplasm is usually abundant staining a darker blue with Wright's stain than do normal large lymphocytes. The cytoplasm contains fine or coarse azure granules and one of the most striking features is the vacuolation. The vacuoles are of two types they may be large and scattered in cells with abundant cytoplasm or they may be smaller and more numerous. The combination of fine vacuolation and basophilia gives the cytoplasm a distinctive foamy appearance.

The nucleus of the cells in patients with infectious mononucleosis may be round oval or kidney shaped occasionally it is divided and often eccentrically placed. The Downey type I cell refers to the cells with dark blue foamy cytoplasm and an irregular kidney shaped nucleus. The Downey type II cell possesses larger amounts of light blue cytoplasm and an eccentrically placed nucleus of normal appearance. The diagnosis in the acute stages of infectious mononucleosis is made by the presence of one or another of the characteristic types of cells and by the assortment of unusual cells in the blood smear.

Occasionally small numbers of large rounded cells with well differentiated clear nuclei and lightly basophilic cytoplasm resembling reticuloendothelial cells appear in the peripheral blood. They probably originate from areas of reticulum cell proliferation in affected lymph nodes.<sup>10</sup>

Just as agglutination may appear in higher titer in patients with diseases other than infectious mononucleosis so have these atypical cells been noted in patients with a variety of conditions such as infectious hepatitis virus pneumonia German measles measles acute brucellosis influenza roseola infantum and others.



Fig. 34 Infectious mononucleosis. 1. Photomicrograph of several types of atypical lymphocytes found in the blood smear of patients with infectious mononucleosis. A and C possess a finely vacuolated cytoplasm giving a foamy appearance, exaggerated in C by the deep basophilia. The color of the cytoplasm with Wright's stain varies from a slate color to the deep blue of a plasma cell. The nuclear pattern in B is not so coarse as that in the other cells and is more immature, with finer strands of lighter staining chromatin. Several nucleoli are present. This cell resembles somewhat the lymphoblast and is often referred to as the Downey type III cell (see A in Fig. 34-2) (Courtesy Dr. Ralph L. Engle, Jr., New York, N.Y.).

2. Drawings of representative cells in the blood smear of patients with infectious mononucleosis. Although a number of different cell types usually appear in the blood smear at one time, especially at the height of the disease, all of these cells were not found together in any single case. F is a cell with irregular edges, eccentric nucleus, abundant cytoplasm, and concentration of basophilia in the periphery. A lightly staining perinuclear zone is observed in A. Deep basophilia of the cytoplasm is noticeable in G, H, and K. Vacuolation of the cytoplasm is present in E and G. The finer vacuolation and deep basophilia in G give the cytoplasm a foamy appearance, an important diagnostic feature. Cells of type K occurred most infrequently. The nucleus of the K type cell stains lightly and approaches in immaturity the nucleus of the lymphoblasts. (From Smith, C. H., Infectious Lymphocytosis, *Am. J. Dis. Child.* 62:31, 1941.)

Because of the increased incidence in so-called virus diseases the atypical lymphocytes have been termed *virocytes*.<sup>3,4</sup> It is important to point out that the blood smears of young infants are particularly prone to show these cells in a number of nonspecific infections unrelated to infectious mononucleosis. In patients with conditions other than infectious mononucleosis however the abnormal mononuclear cells appear in much smaller numbers rarely reaching 10 per cent. At the height of the illness this quantitative difference serves as a differential criterion. Eosinophilia has been observed during the acute stage of the disease but is even more common during convalescence.

It has already been stated that the blood picture in the introductory stages may simulate leukemia. This confusion results from the occasional presence of immature cells resembling lymphoblasts (Downey type III cells) especially when they are observed in association with anemia, leukopenia and thrombopenia. However the eventual appearance of atypical lymphocytes in significant numbers provides an indication of the true nature of the process.

The red blood cell count, hemoglobin concentration, platelet count, sedimentation rate, coagulation time and bleeding time are usually normal. A mild anemia may attend convalescence. Exceptionally, thrombocytopenic purpura<sup>6,9</sup> and acute hemolytic anemia<sup>10,11</sup> have been reported during the course of infectious mononucleosis.

Acute hemolytic anemia occurring during the course of infectious mononucleosis is often associated with marked prostration, high fever and deepening jaundice. The anemia may be of sufficient severity to necessitate splenectomy.<sup>4</sup> Uncommonly, a positive antiglobulin (Coombs) test,<sup>12</sup> cold agglutinins<sup>13</sup> and autohemolysis indicative of an autoimmune mechanism have been present.

It has been postulated that the etiologic factors, viral or bacterial, responsible for causing infectious mononucleosis may also injure the red cells or platelets directly or through antibody formation. Abnormal splenic function or hypersplenism may be involved similarly in reducing any of the formed elements of the blood in patients with infectious mononucleosis inasmuch as this disease is so often accompanied by splenomegaly.

**Heterophil Antibody (Paul Bunnell) Test.** Although there is no unanimity of opinion, a heterophil antibody titer of 1:112 is usually considered diagnostic although the critical level stated in various laboratories ranges from 1:56 to 1:1792. No correlation appears to exist between the heterophil antibody titer and the severity of the disease. The heterophil antibody test has been found positive in 60 to 90 per cent of patients and for that reason examination of the blood smear for atypical lymphocytes is still of paramount importance. The timing of tests is important since the titer may be low in the first week and usually reaches a peak in the second and third weeks in some instances to extraordinary levels. An elevated titer may persist during convalescence usually two to five months, sometimes six months and longer after the symptoms subside. It may revert to normal however in six to eight weeks. Repetition of tests therefore is important in doubtful cases and increased positive reactions probably depend upon this factor. When venous blood is difficult to obtain, a fingertip microheterophil agglutinin

technique has been described which compares favorably with the regular heterophil technique<sup>16a</sup>

According to Vahlquist and associates,<sup>46</sup> a positive Paul Bunnell reaction is rare below 5 years of age and this is explained by a defect in antibody formation during this period. Past this age the reaction does not differ from adult cases with respect to intensity and duration of the serologic reaction.

**Differential Agglutination Tests** Heterophil agglutinins against sheep cells are present in low titer in normal serum and after the injection of horse serum. These are the Forssman type of antibodies which are distinct from those elaborated in patients with infectious mononucleosis. The fact that the Forssman type of antibody combines actively with guinea pig kidney and does not react with beef red cells is employed in the diagnosis of infectious mononucleosis. The heterophil antibodies present in patients with serum sickness and in those with normal serum can be removed by absorption with guinea pig kidney, whereas antibodies in the serum of patients with infectious mononucleosis are not absorbed to the same extent. Although complete absorption with beef red cells is to be expected in patients with infectious mononucleosis, if at least one-fourth of the agglutinin titer remains after absorption with guinea pig kidney, the test is positive for infectious mononucleosis.<sup>6</sup>

Table 16 indicates the scheme of differential agglutination.

*Table 16 Scheme of Differential Agglutination*

| Source of Serum          | Heterophil Agglutinins (after absorption by) |            |
|--------------------------|----------------------------------------------|------------|
|                          | Guinea Pig Kidney                            | Beef Cells |
| Infectious mononucleosis | Present                                      | Absent     |
| Normal serum             | Absent                                       | Present    |
| Serum sickness           | Absent                                       | Absent     |

Absorption tests therefore add to the accuracy of the diagnosis, especially in patients with low titers. In clinical practice, however, these tests are frequently not undertaken when the blood smear and supportive clinical features confirm the diagnosis. In the presence of other unequivocal evidences of this disease, a negative serologic test does not rule out the diagnosis of infectious mononucleosis.

Absorption tests are especially pertinent in rare instances in which the Paul Bunnell test has been found positive without the associated features of infectious mononucleosis. These include a variety of conditions in which an occasional patient may reveal a significant titer—for example, infectious hepatitis, purpura, German measles, scarlet fever, sarcomas, upper respiratory infections, primary atypical pneumonia, monocytic and myelogenous leukemia, thrombocytopenic purpura,<sup>42</sup> and Hodgkin's disease.<sup>36</sup> In one series<sup>40</sup> elevated heterophil antibody titers (1:112 or more) were observed in 19 per cent of patients with acute leu-

leukemia This antibody was completely or almost completely absorbed by guinea pig kidney demonstrating its relationship to the Forssman type in contrast to poor absorption in patients with infectious mononucleosis No elevated heterophil antibodies were observed in this series in patients with chronic leukemia

A sharp reduction in titer has been observed when enzyme (papain) treated sheep erythrocytes are mixed with the serum of patients with infectious mononucleosis In contrast the serum of patients with an elevated heterophil antibody titer unrelated to infectious mononucleosis showed a conspicuous increase in reactivity with enzyme treated sheep red cells as compared with their reactivity with untreated cells

We have noted unabsorbed titers ranging from 1:112 to 1:7168 in patients with thrombocytopenic purpura severe Cooley's anemia lymphoma and cirrhosis of the liver and in children with regional ileitis

Recently an ox cell hemolysin test has been checked against the heterophil antibody test in patients with infectious mononucleosis and a degree of sensitivity was found to justify further study<sup>1</sup>

**Differential Diagnosis** The diverse clinical picture the wide range of symptoms and the pathologic lesions in patients with infectious mononucleosis may obscure the diagnosis and require a consideration of many diseases which it mimics These include leukemia acute infectious lymphocytosis poliomyelitis German measles chronic upper respiratory infections rheumatic fever acute appendicitis infectious hepatitis pertussis mumps influenza and lymphocytic choromeningitis Usually the enlargement of the lymph nodes and the spleen sore throat, fever numerous atypical lymphocytes in the blood smear an increased titer of heterophil antibodies and the absence of anemia and of invasive blast cells in the marrow establish the proper diagnosis Although infectious hepatitis and infectious mononucleosis resemble each other by liver function tests and in pathologic findings infectious mononucleosis usually is associated with an increased heterophil antibody titer and the number of patients with clinical jaundice is small

It should also be pointed out that during the first months of life small numbers of lymphocytes occasionally are observed in the blood smears from normal infants which resemble the abnormal cells found in patients with infectious mononucleosis These cells however bear no relationship to this disease or to any other related condition

**Prognosis** In spite of the alarming nature of many of the symptoms the prognosis is good except in rare patients with splenic rupture or infections Fatal cases of infectious mononucleosis have occurred however without rupture of the spleen They reveal a widespread involvement of lymphoid tissue and the presence of atypical mononuclear cells throughout a majority of the organs<sup>27</sup>

**Treatment** The disease is self limited and no specific therapeutic measure has proved effective Treatment is symptomatic and reliance must be placed upon rest and supportive measures in view of involvement of various organs especially the liver testes, spleen and heart

In children infectious mononucleosis is rare although the overall incidence in all age groups has been approximately 5 per cent<sup>28</sup> Artificially may be of value in

patients with severe sore throat and in controlling secondary infections. Recently ACTH and cortisone have been employed in diminishing the severity of the disease<sup>7</sup> in respiratory distress<sup>12</sup> in relieving allergic manifestations and in the treatment of meningoencephalitis.<sup>13</sup> It should be emphasized that the value of the steroid hormones in reducing constitutional symptoms must be balanced against the risk of disseminating a concomitant or occult bacterial infection.<sup>9</sup>

For the patients with hemorrhagic manifestations due to thrombocytopenia treatment with the adrenocortical steroids has been effective. Prednisone or prednisolone is given in a dosage of 5 to 10 mg every six hours to children under the age of 10 years with a higher dosage ranging to a maximum of 20 mg every six hours in the adolescent patient. Treatment for five to seven days should effect an increase in platelets and in the control of hemorrhage.<sup>48</sup> In very unusual cases splenectomy is necessary to control hemorrhage due to thrombocytopenia. For patients with the rare complication of hemolytic anemia transfusions are given with or without the steroid hormones. In one patient in whom cortisone was ineffective splenectomy was resorted to with a favorable response.<sup>44</sup>

**Recurrences** Although unquestionable recurrences have been reported they are extremely rare and frequently fail to demonstrate significant heterophil antibodies. However a nonspecific rise in titer occurs some months after the initial attack especially following an upper respiratory infection. Since atypical lymphocytes are known to persist for extended periods recurrences may be erroneously diagnosed. More extensive experience is required with a combination of laboratory techniques especially liver function tests to rule out the influence of hepatitis as a causative factor in prolongation and chronicity of the disease or its recurrence.

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## *Leukemia—General Aspects and Clinical Features*

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Leukemia is a disorder of the white blood cells of unknown etiology with a fatal outcome. It is generally considered to be a neoplastic change in the blood-forming tissues and is characterized by an uncontrolled and disorderly proliferation of leukocytes and their precursors with invasive tendencies. Although the white blood cells are usually present in excessive numbers in the circulating blood, they may be present in normal or reduced numbers. In either case, immature or abnormal cells are present during a major part of the course of the disease.

It is of interest historically that, although cases of leukemia had been previously reported by others, credit for ascribing the altered state of the blood to an increase in the colorless cells rather than to pus and for naming the condition leukemia (white blood) goes to Virchow.<sup>\*</sup>

Furth<sup>34</sup> has emphasized the essential identity between leukemia and neoplasms since in both conditions the immature cell has acquired an inability to respond to forces in the host normally regulating their maturation and proliferation. Many concepts, especially those relating to causation and treatment of leukemias in human beings, stem from animal experimentation, especially from experiments on leukemia in mice which is similar both pathologically and clinically to that in human beings.

No proof exists yet that leukemia in mice is identical with that in human beings, although similar effects result in increasing susceptibility following ionizing radiation and in parallel therapeutic responses following administration of a variety of chemical agents. New mitotic poisons employed in the treatment of human patients have been obtained by screening in mice with experimentally produced leukemia.

**Classification of Leukemia in Childhood** Leukemia is classified as acute, subacute, or chronic and by the type of white blood cell predominating in the bone marrow and circulating blood. No rigid criteria can be applied for designating the exact duration of the disease. It is much more practical, especially since survival has been extended by chemotherapy, to employ the terms acute and chronic in relation to clinical severity and to qualify further as to the type of cell or cellular pattern predominating in the disease.

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**Acute Stem Cell Leukemia** The predominant cell in the bone marrow and peripheral blood in the majority of patients with acute leukemia is usually primitive and there is a virtual absence of cellular differentiation into myeloblastic or lymphoblastic types. The morphologic characteristics in these patients do not suggest any specific line of development. Although it is possible in rare cases to separate these immature cells the term stem cell leukemia overcomes the difficulties of differentiation. The majority of these cells are often classified as lymphoblasts because of their resemblance to lymphocytes in their proximity. It is for this reason that acute lymphoblastic leukemia frequently has been regarded as the most common form of leukemia in childhood. (For differentiation of cell types occurring in leukemia see Chapter 18) In the more recent reports the terms granulocytic and lymphocytic are used for the leukemias instead of myelogenous and lymphatic.

**Granulocytic Types** Granulocytic types of leukemia encountered in children are of three types: acute promyelocytic, acute myelocytic, and chronic myelocytic or myelogenous.

**ACUTE PROMYELOCYTIC LEUKEMIA** Acute promyelocytic leukemia, the most frequent member of the granulocytic group, is a disease in which the predominant cells are early myelocytes (myelocytes A and B). The terms acute myeloblastic leukemia and acute promyelocytic leukemia sometimes are used interchangeably in this type because either blast forms or promyelocytes may predominate at one time or another in the same patient. However, in contrast to the myeloblast which is devoid of granules, a few darkly stained granules are found in the promyelocyte. Cases reported as acute granulocytic leukemia probably fit into this category.

**ACUTE MYELOCYTIC LEUKEMIA** In patients with acute myelocytic leukemia the blood smear and especially the bone marrow are characterized by the presence of more mature granules than those of the promyelocytic form. The cells possess numerous granules scattered throughout the somewhat grayish or basophilic cytoplasm. Because of the greater number of granules and the more advanced maturity this type of cell is often termed myelocyte C in contrast to less mature myelocytes A and B containing fewer granules characterizing the promyelocytic form. Acute myelocytic leukemia is differentiated from the chronic myelogenous type not only by the more moderate leukocytosis but also by the narrow range of maturity of the abnormal forms. One of the features of acute myelocytic leukemia is the cytologic resemblance in many of the cells to the monocyte, especially with regard to the nucleus.

**CHRONIC MYELOCYTIC (GRANULOCYTIC MYELOID, OR MYELOGENOUS) LEUKEMIA** Chronic leukemia is infrequent in childhood, being confined to the chronic myelocytic type which accounts for about 2 per cent of all leukemias in this age period. Hyperleukocytosis is common with segmented and nonsegmented neutrophils constituting 75 per cent of the total. Myelocytes and metamyelocytes are present in increased numbers. The bone marrow may show larger numbers of myelocytes than the peripheral blood although samples from both areas are characteristically similar. Relatively few myeloblasts or immature myelocytes are found at any time except terminally. Basophils are increased and platelet counts

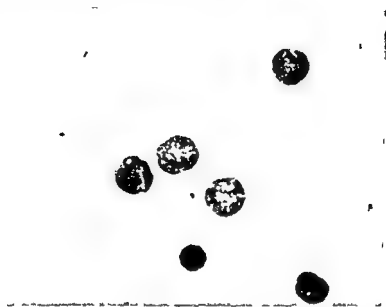


Fig. 35 Photomicrograph from blood smear of a patient with acute leukemia. Note lighter staining and finely granular or stippled appearance of nucleus in lymphoblasts in upper section. Compare with dark staining by chromatin of nuclei of two normal lymphocytes in lower section ( $\times 1200$ ).

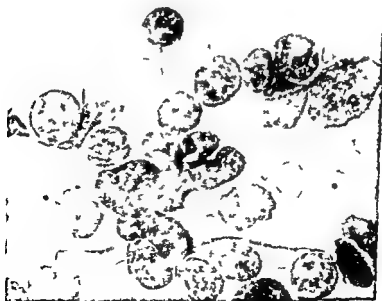


Fig. 36 Photomicrograph of bone marrow smear from a patient with acute leukemia ( $\times 1200$ ). Note uniform infiltration by lymphoblasts.

**Acute Stem Cell Leukemia** The predominant cell in the bone marrow and peripheral blood in the majority of patients with acute leukemia is usually primitive and there is a virtual absence of cellular differentiation into myeloblastic or lymphoblastic types. The morphologic characteristics in these patients do not suggest any specific line of development. Although it is possible in rare cases to separate these immature cells the term stem cell leukemia overcomes the difficulties of differentiation. The majority of these cells are often classified as lymphoblasts because of their resemblance to lymphocytes in their proximity. It is for this reason that acute lymphoblastic leukemia frequently has been regarded as the most common form of leukemia in childhood. (For differentiation of cell types occurring in leukemia see Chapter 18) In the more recent reports the terms granulocytic and lymphocytic are used for the leukemias instead of myelogenous and lymphatic.

**Granulocytic Types** Granulocytic types of leukemia encountered in children are of three types: acute promyelocytic, acute myelocytic, and chronic myelocytic or myelogenous.

**ACUTE PROMYELOCYTIC LEUKEMIA** Acute promyelocytic leukemia, the most frequent member of the granulocytic group, is a disease in which the predominant cells are early myelocytes (myelocytes A and B). The terms acute myeloblastic leukemia and acute promyelocytic leukemia sometimes are used interchangeably in this type because either blast forms or promyelocytes may predominate at one time or another in the same patient. However, in contrast to the myeloblast which is devoid of granules, a few darkly stained granules are found in the promyelocyte. Cases reported as acute granulocytic leukemia probably fit into this category.

**ACUTE MYELOCYTIC LEUKEMIA** In patients with acute myelocytic leukemia, the blood smear and especially the bone marrow are characterized by the presence of more mature granules than those of the promyelocytic form. The cells possess numerous granules scattered throughout the somewhat grayish or basophilic cytoplasm. Because of the greater number of granules and the more advanced maturity, this type of cell is often termed myelocyte C in contrast to less mature myelocytes A and B containing fewer granules characterizing the promyelocytic form. Acute myelocytic leukemia is differentiated from the chronic myelogenous type not only by the more moderate leukocytosis but also by the narrow range of maturity of the abnormal forms. One of the features of acute myelocytic leukemia is the cytologic resemblance in many of the cells to the monocyte, especially with regard to the nucleus.

**CHRONIC MYELOCYTIC (GRANULOCYTIC MYELOID OR MYELOGENOUS) LEUKEMIA** Chronic leukemia is infrequent in childhood, being confined to the chronic myelocytic type which accounts for about 2 per cent of all leukemias in this age period. Hyperleukocytosis is common with segmented and nonsegmented neutrophils constituting 75 per cent of the total. Myelocytes and metamyelocytes are present in increased numbers. The bone marrow may show larger numbers of myelocytes than the peripheral blood, although samples from both areas are characteristically similar. Relatively few myeloblasts or immature myelocytes are found at any time except terminally. Basophils are increased and platelet counts

are either normal in number or may reach levels of 1 million per cubic millimeter or more. In patients with chronic myelogenous leukemia the neutrophils contain very little alkaline phosphatase, whereas in those with pyogenic infections associated with a leukocytosis or a leukemoid reaction corresponding cells exhibit a great increase in this enzyme.<sup>194a, 101</sup> Auer bodies rod shaped structures in the cytoplasm which are peroxidase positive may be present in the myeloblasts or myelocytes. As the disease progresses anemia and thrombocytopenia prevail as in patients with the acute leukemias.

The disease is rare in childhood but is often seen in the first months of life as a form of congenital leukemia. In affected infants the cells are as well differentiated as they are in patients with the adult form of the disease but in contrast to the prolonged survival in the latter the course in the infant is short. The older child may survive for a period of several years. Nevertheless the designation chronic myelocytic leukemia in the young patient refers to the type of blood picture rather than to the length of the survival.

The onset of the disease is particularly difficult to designate in patients with chronic myelocytic leukemia because the symptoms are vague. A routine blood count made because of continued low grade fever and fatigue frequently calls attention to an underlying hematologic disorder. The marked leukocytosis with a shift of the neutrophils to the left and the presence of a few metamyelocytes and myelocytes suggest a diagnosis of a leukemoid reaction initially. Hepatosplenomegaly and lymphadenopathy are usually marked in patients with this form of leukemia. At the onset these features may be absent except for a palpable spleen which enlarges as the disease progresses. Pain about the hip may become so pronounced that walking is painful. The marked limp which develops early rarely correlates with an abnormal finding in the roentgenogram.

Monocytic Leukemia Monocytic leukemia is similar to the other types in most respects. The onset may be insidious with a fatal outcome in a few weeks or months or it may be sudden with the disease running its course within a few days. In patients with the latter type of onset the spleen is slightly enlarged and the liver and lymph nodes may not be palpable. The disease has an age incidence ranging from early infancy to later childhood. The symptoms and physical signs are similar to those of other types of leukemia except that hypertrophied and spongy gums have been described more frequently in patients with the monocytic type.

Two types of monocytic leukemia are described. When the blood picture is marked by the simultaneous presence of both myelocytes and monocytes or immature cells intermediate between myeloblasts or myelocytes and monocytes the disease is designated as the Nieglh type or myelomonocytic leukemia. When the immature cells resemble monocytes and reticuloendothelial cells the disease is designated as the Schilling type. (See Chapter 18.)

Chronic monocytic leukemia is extremely rare in childhood. Only two patients in this category were observed in a series of 900 cases of childhood leukemia.<sup>1</sup> The disease may be difficult to diagnose in the early stage because of predominant megaloblastic blood and bone marrow changes, diverse skin lesions and spontaneous remissions. However the finding of varying numbers of cells resembling



adult monocytes and their precursors in the peripheral blood and bone marrow should arouse suspicion of the underlying disease. Early in the course of the disease there may be a marked leukocytosis or a leukopenia.

**Eosinophilic Leukemia** Eosinophilic leukemia is an acute disease of childhood in which the majority of cells in the peripheral blood and tissues are mature eosinophils but in some cases are associated with myeloblasts and myelocytes. Enlargement of the liver, spleen and lymph nodes, anemia and thrombocytopenia may not be present until late in the disease. The blood picture may change to that of an acute myeloblastic leukemia but more often tissue infiltration and the peripheral blood disclose invasion by the adult eosinophil. The slight elevation in the number of myeloblasts occasionally encountered is not sufficient for the diagnosis of acute leukemia.<sup>18, 39</sup>

In a 11 year old boy with eosinophilic leukemia the leukocytes which numbered 88 000 per cubic millimeter of blood (80 per cent mature eosinophils) were associated with diffuse parenchymal infiltration of both lungs and intermittent fever. In this patient steroids (prednisone) produced a temporary regression of lung infiltration and symptomatic relief. Postmortem examination revealed widespread tissue infiltration with mature eosinophils.

In terms of clinical severity stem cell promyelocytic monocytic chronic myelocytic and eosinophilic leukemias are acute leukemias in childhood. Chemotherapy and other modern supportive measures however have modified their course and prolonged life. It should be borne in mind that acute stem cell leukemia is essentially a disease of infancy and childhood although no age group is spared. In rare instances chronic myelocytic leukemia in children will run a prolonged course simulating that in the adult. Aleukemic leukemia is the designation for leukemia in patients in whom there is either no leukocytosis or a marked reduction in leukocytes but usually with the presence of abnormal cells in the peripheral blood and tissue histology showing the true nature of the disease. Chronic lymphocytic leukemia is the most protracted and benign type of leukemia and occurs invariably in adults.

**Incidence Age Distribution Sex and Frequency of Types** Acute leukemia is commonest between 2 and 5 years of age with the incidence rising from a moderate elevation in the first two years of life to a peak in the third and fourth years.<sup>1</sup> Although acute leukemia predominates in the first four to five years of life myelocytic leukemia is the commonest form of the chronic disease up to the age of 60 years after which the lymphocytic type is most frequently encountered. In total numbers chronic myelocytic leukemia and chronic lymphocytic leukemia are more frequent in the groups over 50 years of age.<sup>1, 37</sup>

According to Cooke<sup>1</sup> there is a gradual increase in the proportion of males to females who have acute leukemia with the predominance of boys being greater in later childhood than during infancy. In the first year of life more cases are observed in girls than in boys with this exception leukemia is more commonly observed in the male than in the female at all other ages.

Mortality statistics show that there has been a progressive increase in the death rates from leukemia. In patients in the youngest age group (0 to 4 years) the rate

has risen from 2.2 to 5.1 per 100 000 deaths between 1930 and 1949.<sup>4</sup> In one study<sup>58</sup> the incidence in one large hospital from 1932 to 1948 was 0.42 per cent of all admissions to the general pediatric service. Figures showing the changing incidence of leukemia cannot be truly ascertained from hospital admissions alone since there is a tendency for patients with leukemia, especially in the younger age groups, to be concentrated in large hospitals and medical centers. However the recorded death rate from leukemia at all ages has risen steadily in the United States from 1.4 in 1921 to 6.3 in 1953.<sup>5</sup> An over all mortality rate from leukemia of approximately 10 per 100 000 has been estimated for the United States.<sup>5,10</sup> In recent years a similar increase in all types of leukemia has also been noted in England and Wales. In Scotland an approximate doubling of the incidence of admissions for leukemia to several large hospitals was recorded during the period from 1938 to 1951.<sup>58</sup>

Within six months we have observed three unrelated children under the age of 5 years with acute leukemia living within one half mile of each other. Intensive investigation however revealed no known leukemogenic agent to which the three children had been exposed. This relationship in so many aspects seemed a startling experience and may eventually prove to be more than coincidental.

**Etiology** Various physical, chemical and metabolic agents have been found capable of precipitating or activating the development of leukemia. Infectious agents, trauma,<sup>103</sup> and exposure to drugs and chemicals have appeared to be so closely associated with leukemia as to suggest a causative relationship. Arsenicals, sulfonamides and benzol poisoning<sup>60</sup> have been prominently mentioned but evidence is still incomplete. The widespread use of antimicrobial and antibiotic agents in the treatment of patients with infectious disease has led to the impression that they may possess leukemogenic activity although there has been no proof of this relationship.

The morphologic analogy between the maturation arrest in the bone marrow of patients with pernicious anemia and the blast cells in those with acute leukemia has suggested that leukemia represents a metabolic or deficiency disease but treatment on this basis has given negative results.<sup>59</sup> In another theory leukemia is based on disordered hormonal or metabolic functions. In one group of experiments<sup>64</sup> specific substances that produce myeloid and lymphoid proliferation and maturation in animals have been extracted from the urine of patients with leukemia. Data have also been obtained supporting the concept that the failure of cell maturation in patients with leukemia is associated with a loss of factors which inhibit cell proliferation.<sup>6</sup> Leukemia has also been compared to a type of autoimmunization involving the proliferation of one of the white cell forming tissues in which the response once begun is self replicating and permanent.<sup>30a</sup> The stimuli for such leukemogenic activities are ionizing radiation, carcinogenic chemicals and viruses.

**Infections** The role of infections in the causation of leukemia receives periodic emphasis. Cooke<sup>1</sup> has pointed out that infection frequently antedates the development of acute leukemia in childhood and that the character of the age incidence for leukemia tends to parallel the frequency of acute infections. One of the difficulties in accepting the infectious concept of leukemia however has

been the well-established observation that in human beings with leukemia, as in experimental animals with leukemia there is the lack of communicability which characterizes other infectious diseases

**Viral Etiology** With the possibility that nonspecific infections conceivably may be causally related to acute leukemia of childhood a number of studies have focussed their attention on a viral etiology for human leukemia. The demonstration by Ellerman and Bang<sup>31</sup> that chicken lymphomatosis could be transmitted by filtered extracts stimulated interest in the causation of leukemia and allied diseases in other species as well as in human beings. Cross<sup>32</sup> demonstrated a viral etiology for lymphoid leukemias in mice which is noncontagious but is communicated to offspring during embryonal life through either the male or the female parent resulting in leukemia after a period of latency of one third to one half of the life span of the animal. According to this hypothesis leukemia develops when the dormant agent is activated any time during the life span of the mouse. The viral agent thus exists in inactive form manifesting malignant capabilities only when challenged by as yet obscure but presumably trigger stimuli such as x rays, chemicals, infections or products of endogenous metabolic activities. Also the virus would not necessarily become activated during the life span of a single carrier host but would be transmitted from one generation to another in a vertical pattern.

Schwartz and Schoolman<sup>33</sup> extended these observations to show that cell free filtrates prepared from the brain of patients who died from acute leukemia are capable of accelerating the development of or inducing this disease in a leukemic strain (AAR) of mice. These studies based on animal experimentation are provocative and provide a basis for explaining many of the features of human leukemia seen in clinical practice. The confirmation for this hypothesis in human leukemia however awaits the discovery of the virus in man. A detailed comparison between any type of human leukemia with the response of an inbred animal strain to any of the leukemogenic virus has still to be justified.<sup>34</sup>

**Heredity** The agents found to be leukemogenic or to accelerate the onset of leukemia in mice depend upon the genetic constitution of the mouse.<sup>35</sup> In the human being as well attention has been drawn to the possibility that the genetic constitution may condition susceptibility to leukemia. Many instances have been reported in which leukemia developed simultaneously in several members of the same family or in successive generations. The most striking family history is that reported by Anderson<sup>3</sup> in which five out of eight siblings developed leukemia at 5 to 8 years of age. The occurrence of leukemia in twin children especially in identical twins has been the subject of several reports.<sup>36-41</sup> Gunz and Dameshek<sup>41</sup> reported a family in which a man aged 53 years died of chronic lymphocytic leukemia twenty seven years after his father and the father's identical twin died of the same disease both at the age of 56 years.

Analysis of large series of cases in any one hospital provides little evidence however that heredity is of any importance in human leukemia. On the other hand in the light of experimental and unequivocal evidence of multiple cases in one family the hereditary factor cannot be categorically dismissed. Videbaek<sup>42</sup> for instance finding a familial incidence of leukemia of about 8 per cent in

Denmark as compared with 0.5 per cent in control families concluded nevertheless that leukemia was a matter of an inherited predisposition rather than of direct chromosomal inheritance.

Leukemia has never been known to pass the placental barrier either in the mouse<sup>16</sup> or in the human being. In the experimentally produced disease the leukemic cells are found only on the maternal side of the placenta, the fetal circulation remaining normal. Experience has been universal that leukemic mothers give birth to normal infants, the placenta apparently serving as an effective barrier.<sup>10</sup>

A probable exception to this observation is the recent report of a mother with signs and symptoms of leukemia beginning in the seventh month of pregnancy in whom acute lymphocytic leukemia was diagnosed eight days postpartum and whose infant developed the same disease at 11 months of age.<sup>6</sup> The problem here is one of establishing an absolute relationship between leukemia in the mother and infant because of the lapse of time until the disease was noted. If transplacental passage of the agent occurred late in pregnancy it is theoretically possible for the disease not to have become manifest until some time after birth.<sup>6</sup>

**Radiation.** In addition to the long term genetic effects of ionizing radiation<sup>47,48</sup> there is mounting evidence that this agent is strongly implicated in the causation of leukemia and is a major factor in the steadily increasing death rate from this disease. The evidence for irradiation as a leukemogenic agent in human beings includes the increased incidence of leukemia among radiologists as compared with other physicians<sup>61</sup> in patients receiving radiation therapy for ankylosing spondylitis<sup>1</sup> in children who received radiation to the mediastinum for enlarged thymus in infancy,<sup>62,63</sup> and among atomic bomb survivors in Hiroshima and Nagasaki.<sup>33,64,65</sup> Hematologic surveys in Hiroshima indicated that after atomic radiation there was a "latent period" of several years before leukemia was in evidence. Chronic myelogenous leukemia was most common, followed in frequency by acute myelogenous and acute lymphatic types.<sup>66</sup>

It has been postulated that the increased exposure of the population to ionizing radiations employed in medicine and dentistry also contributes to the accelerated death rates from leukemia.<sup>6</sup> There is a possibility that the spontaneous incidence of leukemia may be due to radiation from natural background sources. Quantitative estimates have been made of the probability of developing radiation induced leukemia in the average person in a population.<sup>6</sup>

It is of interest that age has been shown to be a factor of importance in experimentally induced leukemia. Animals exposed to whole body irradiation before puberty develop a far higher incidence than older animals.<sup>6</sup> Besides causing genetic damage, diagnostic pelvimetry during pregnancy or threatened abortion may occasionally cause leukemia or cancer in the unborn child.<sup>6</sup>

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In the light of these concepts it would appear that certain genetic influences acting in conjunction with both identified and as yet unidentified external factors transform normal hematopoietic cells and their precursors into those with an unrestrained and purposeless growth.<sup>62</sup> Exposure to ionizing radiation in the environment, infections, contamination of the atmosphere with chemical pol-

lutants and marrow depressings drugs and chemicals may serve as such activating agents.<sup>60</sup> Despite these incriminating data for the carcinogenic effect of radiation it is not possible yet in man to determine whether or not a threshold dose for the induction of leukemia does or does not exist.<sup>61</sup>

**Preleukemic Stage of Leukemia** Cases of leukemia have been recorded in which a nonleukemic blood disorder of variable duration preceded the onset of acute leukemia.<sup>3</sup> This preliminary period is usually characterized by a deficiency of one or more cellular elements in the peripheral blood resulting in anemia, neutropenia, thrombocytopenia, or combinations thereof. The bone marrow is moderately hyperplastic and may or may not show an arrest in the granulocytic series.<sup>18</sup> In one patient the preleukemic stage extended over a period of nine years and was punctuated by a remission before the terminal development of myeloblastic leukemia.<sup>100</sup>

The anemia is frequently hemolytic in nature although not always accompanied by an elevated reticulocyte count.<sup>6</sup> In one case in our experience acute stem cell leukemia in a child 4 years old was preceded two years earlier by severe iron deficiency anemia. Neither the blood nor bone marrow showed evidence of immature white blood cell precursors. In the intervening period the child was asymptomatic without changes in the blood status. In children prolonged neutropenia and leukopenia or aplastic anemia may appear to precede acute leukemia but when this sequence is evaluated further it appears possible that they represent a remission phase in the course of the latter disease. Abnormal cells indicative of leukemia are not found in the blood or bone marrow of patients with these antecedent disorders and there is no associated splenomegaly or lymphadenopathy. Still to be determined by a more direct approach than circumstantial evidence is the extent to which the initial disorder is involved in the subsequent development of leukemia.

**Leukemia in the Newborn Period (Congenital Leukemia)** Congenital leukemia is a rare disease of which only forty-five adequately documented cases have been reported.<sup>4</sup> Some infants exhibit signs of leukemia at birth and succumb shortly thereafter. In another group the infant appears normal at birth but develops clinical and hematologic signs later in the newborn period. In the third group the disease is not detected until the third to the sixth week of life,<sup>11a</sup> with a suggestive history of a hematologic disturbance dating back to the first weeks of life.

The leukemia is principally myelogenous and is marked by a hyperleukocytosis and a predominance of promyelocytes and myelocytes. Myeloblasts vary from 10 to 80 per cent. The granulocytic leukemia is noted both in the blood and bone marrow.<sup>9, 7, 1, 2, 75</sup> Anemia is uncommon in the newborn infant but develops soon thereafter with a rapid progression to pancytopenia. Large numbers of nucleated red blood cells may be present in the peripheral blood regardless of anemia. Those cases that develop later show anemia when diagnosed. Platelets are reduced in number. Physical signs include hemorrhages of the skin, bleeding from the mucous membranes and umbilical stump, nodular skin infiltration and enlargement of the liver and spleen.

In the newborn period leukemia is to be differentiated principally from the

leukemoid reaction of sepsis and congenital syphilis erythroblastosis and congenital thrombocytopenic purpura. In patients with a leukemoid reaction myelocytes as well as segmented and nonsegmented polymuclear cells are increased but myeloblasts are absent. Eventually the causative agent of the sepsis is discovered by blood culture or by evidences of specific organ involvement with infection. The essential differentiating feature is the extensive infiltration of the nonhematopoietic organs as well as the liver spleen and lymph nodes by immature cells of the granulocytic series in infants with leukemia and the absence of such infiltration in patients with a leukemoid reaction. Erythroblastosis which may also be accompanied by a leukocytosis anemia, and myelocytes in the peripheral blood can be diagnosed by evidences of blood group incompatibility. Although the decreased number of platelets in patients with congenital thrombocytopenia may have to be considered hepatosplenomegaly is absent and promyelocytes and myelocytes do not appear in the blood smear.

It is of interest that no case of congenital leukemia has ever been encountered in the newborn infant whose mother had leukemia during pregnancy. Furthermore leukemia diagnosed soon after birth must have had an intrauterine origin without affecting the mother.

Treatment of leukemia with current methods (see Chapter 23) is ineffective and the duration of life varies from several days to two or three months. This rapidly fatal course is in contrast to the prolonged course of chronic myelogenous leukemia in adults and in children beyond infancy.<sup>3</sup>

The presence of associated cardiac orthopedic skeletal and other developmental anomalies in patients with congenital leukemia<sup>9</sup> is analogous to the well documented combination of leukemia and mongolism.<sup>6,9,10</sup> The concept which attributes mongolism to toxic factors of endogenous or exogenous origin inducing changes in the fetus during the sixth to the ninth week of pregnancy<sup>10</sup> may apply to both sets of circumstances with simultaneous and parallel injury to the developing bone marrow. In each instance the occurrence of a predominantly myeloid form of leukemia suggests that the granulocytic precursors rather than the lymphoblasts were principally affected.

**Spontaneous Remissions** Complete and spontaneous clinical and hematologic remissions have been observed in some patients without apparent relation to therapy. They have also occurred in patients on supportive therapy including transfusions or without antibiotics and especially following infections. An incidence of spontaneous remissions as high as 10 per cent of cases has been given in one series in children<sup>10</sup> and as low as only 1 in 105 children with transfusions as the sole therapy.<sup>10</sup> Remissions following infections have been described more frequently. Remarkable improvement and complete and dramatic arrest of the disease have been reported in a group of children with acute leukemia following infection caused by streptococcus staphylococcus and varicella and by inoculation with the feline panleukopenia virus.<sup>11</sup> Four spontaneous remissions were observed in a young child over a seventeen month period on supportive therapy and antibiotics. Characteristic of the majority of cases was an antecedent stage of severe leukopenia frequently a leukopenic agranulocytic phase and hypoplasia or aplasia of the marrow associated with anemia. It is of interest that there

peutically induced remissions also demonstrate a hypoplastic phase preceding remission. This transformation to a normal status is usually short lived, varying from several weeks to a few months, and in one adult reported on complete remission lasted twenty-one months.<sup>1</sup> Following the remission, however, the disease returns to its original severity, uninfluenced by the temporary reversal to normal.

**Clinical Features.** The clinical features of leukemia combine the constitutional manifestations of the disease, blood deficiencies, particularly thrombocytopenia and anemia, and involvement of organ systems.

**Symptomatology.** The clinical manifestations and course of acute leukemia are strikingly similar and show no differences with respect to the predominating cell type. The onset of this disease is insidious, with localized symptoms obscuring the systemic nature of the disease. Symptomatology often follows a well-defined pattern, and several sets of symptoms are observed. Frequently the onset is ill defined, with the patient exhibiting pallor, anorexia, irritability, abdominal pain, and malaise. These symptoms may pass unnoticed until diagnosis by blood examination prompts a retrospective review of the history. Other patients have persistent low grade fever with intermittent periods of moderate or marked temperature elevations which either are unexplained or are attributed to a sore throat. In some the disease is ushered in by high fever and an upper respiratory infection from which the child does not recover as expected with routine treatment. In still others there is the appearance of hemorrhagic manifestations such as bleeding from the gums, epistaxis, and a tendency to bruise. Persistent hemorrhage after a minor operation or after tonsillectomy may be the first evidence of acute leukemia.

More rarely the onset is abrupt with the clinical picture of an acute infectious disease marked by hyperpyrexia, severe prostration, nausea, vomiting, abdominal pain, anemia, and purpura. In a patient with this type of fulminating disease with a duration of several days, the spleen and lymph nodes may be only slightly or moderately enlarged. Symptoms such as local or generalized pain, limp, and bruising dating from a specific injury are sometimes so definitive as to justify a consideration of trauma as an etiologic factor. Migratory bone and joint pains and at times acute arthritis with signs of local inflammation, pallor, weakness, epistaxis, and an irregular febrile reaction simulate the clinical picture of rheumatic fever. Spontaneous rupture of the spleen is a rare occurrence in patients with acute leukemia and may produce the initial symptomatology of the disease.<sup>10</sup>

**Physical Findings.** Early in the course of acute leukemia the physical examination may be essentially normal. Sooner or later the lymph nodes usually enlarge and the edge of the spleen is palpable on deep inspiration. Lymph node enlargement is most marked in the cervical region but may be conspicuous over the occipital and postauricular areas and elsewhere over the scalp. It is often less pronounced than in patients with chronic leukemia. Involvement of the lymphoid tissue resulting in bilateral painless enlargement of the salivary and lacrimal glands is designated as the Mikulicz syndrome.<sup>11</sup> The liver and spleen may be enormously enlarged, but the spleen may fluctuate in size, especially during treatment, with little accompanying change in the liver.

Hemorrhages into the skin consist of petechiae, purpura, and extensive ecchy-

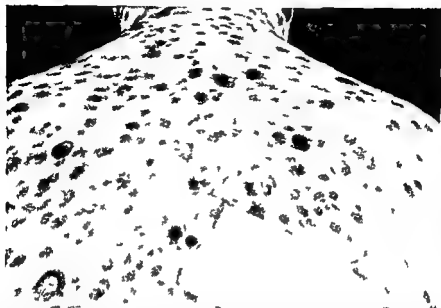


FIG. 37 Skin lesions in child with acute lymphoblastic leukemia (leukemia cutis). The lesions originally discrete were later confluent and covered the face trunk and eventually the entire body. The individual lesions were slightly nodular erythematous and purpuric. Many were eventually necrotic.

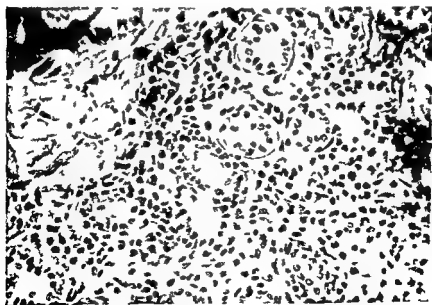


FIG. 38 Biopsy of skin lesion from patient shown in FIG. 37. Note leukemic cells densely infiltrating corium about sweat glands ( $\times 435$ ).



moses which recede and reappear during the disease. Hemorrhages into eye grounds and in mucous membranes especially in the mouth and nose are common. Ulcerations in the buccal mucosa result from leukemic infiltration as well as from hemorrhage.

Swollen spongy hypertrophied gums to the extent that the teeth are hidden which characterizes monocytic leukemia may also be found in patients with acute stem cell leukemia. Ulcerations may extend from the gums to the tonsils and palate.

Skin involvement is varied. It is either specific with leukemic infiltration or nonspecific with erythema multiforme like papuloneurotic and eczematous lesions. More specific are the small yellow discrete and elevated flat topped plaques which are either widespread or confined to localized areas on the abdomen and extremities. Infrequently maculopapular eruptions cover the face initially and spread to the chest and back and eventually to the abdomen and extremities. Each lesion varies from 1 cm or more in diameter may become hemorrhagic and necrotic and is eventually covered by a grayish membrane. Biopsy reveals extensive leukemic infiltration around blood vessels and adnexa in the epidermis and subcutaneous tissue.

Mediastinal involvement should be suspected in the presence of symptoms of respiratory distress. The kidneys are often greatly enlarged and glomerulonephritis may precede or accompany the overt blood changes of leukemia.

**Skeletal Changes.** The encroachment upon actively functioning marrow by leukoblastic cells within the bony skeleton at a period when nonfunctioning yellow marrow is not yet available accounts for the changes observed by roentgenography in patients with this disease. Skeletal lesions and symptoms are less common in adults since they possess large amounts of fatty marrow which can be replaced by leukemic cells before osseous changes occur.<sup>2, 10, 12</sup>

Bone changes consist of transverse bands of diminished density, osteolysis and periosteal elevation with new bone formation. Most commonly multiple areas of destruction and production are seen at variable skeletal sites. The evidence of skeletal lesions varies in different series being given as 50 per cent in one group<sup>4</sup> and 73 per cent in another.<sup>8</sup> Bone pain develops with increased intraosseous tension secondary to the proliferation of the leukemic cells. Less frequently bone pain is caused by infiltration of the synovial membranes of several joints by leukemic cells. These eventually destroy the spongiosa producing local or diffuse rarefaction. Leukemic cells penetrate the cortex of bone and extending beyond it lift up the periosteum which becomes the site of new bone formation. The lifting of the periosteum also results from subperiosteal hemorrhages secondary to thrombocytopenic purpura.

An important diagnostic feature is the marrow translucent zone just proximal to the metaphysis of the shaft of the long bone<sup>6</sup> which is most marked in areas of most rapid growth the distal femur, tibia, radius and ulna in decreasing order of frequency. These zones of diminished density are best demonstrated in the tibia and femur at the knees. Osteosclerosis occurs least frequently and may be either focal or diffuse and is secondary to excessive formation of spongiosa.

The changes observed by roentgenographic examination may be summarized as follows (1) osteoporosis (2) transverse lines of increased density (growth areas) (3) radiotranslucent bands<sup>6</sup> (metaphyseal and epiphyseal) (4) osteolytic lesions (cortical and medullary) (5) cortical thinning and destruction (6) leukemic infiltrations and hemorrhage and (7) periosteal (lifting of periosteum new bone formation)



Fig. 39

Fig. 39 Skeletal lesions of leukemia. Note generalized and local rarefaction and focal areas of bone absorption associated with replacement of bone marrow by leukemic tissue.



Fig. 40

Fig. 40 Arrow points to a narrow, deep transverse zone of rarefaction just proximal to the metaphysis of the long bones, usually most marked in the lower ends of the femurs and tibiae. Note also multiple areas of rarefaction in medullary portion of bones.

**Course** The course of acute leukemia is beset by many complications despite the fact that with chemotherapeutic agents and supportive therapy the patient is free of symptoms for prolonged periods and temporary remissions are induced. The major problems arise from a tendency toward hemorrhage, local or systemic infection and leukemic infiltration of tissues and organs resulting in disturbances from their increased size and interference with function.

**Hemorrhage** The hemorrhagic manifestations of leukemia can usually be ac-

counted for by a reduction in the number of platelets although bleeding need not necessarily occur with it. Why one patient bleeds while another with comparable thrombocytopenia does not is puzzling. The depletion of platelets has been attributed to the depression of megakaryocytes by replacement of bone marrow by primitive cells. Hypoprothrombemia and factor V deficiency due to liver damage have been demonstrated in some patients but there is no consistent relationship with any of these factors. Circulating fibrinolysin resulting in a fibrinogenopenia with massive hemorrhage has also been reported.

The control of bleeding by ACTH and the adrenal steroid hormones without resulting increase in the number of platelets suggests however that vascular dysfunction may result from causes other than thrombocytopenia.

Besides skin involvement with petechiae and ecchymoses serious bleeding can occur in more strategic areas. Intracranial hemorrhage is a frequent terminal complication especially when the number of circulating abnormal cells is high. For this reason the additional hazard of the hypertensive side effects of ACTH and steroids must be watched for during treatment. Epistaxis, hematuria, gastrointestinal hemorrhage and oozing from the gums, ulcerations of the buccal mucous membranes and interruptions in the mucocutaneous junctions occur frequently especially in the later stages of the disease. Episodes of severe bleeding occur repeatedly with extreme degrees of thrombocytopenia in both phases of agranulocytosis and leukemic leukocytosis when the bone marrow is heavily infiltrated. Chemotherapeutic agents such as methoplatin (Methotrexate) administered for prolonged periods or in excessive amounts may produce toxic effects such as ulcerations and bleeding in the buccal mucosa and lips and in the gastrointestinal canal. Necrotic lesions are often present in sites of ulcerated and hemorrhagic areas especially about the mouth.

**Anemia.** Diagnostically the red cells are both normochromic and normocytic and anisocytosis, poikilocytosis and achromia are minimal. Occasionally large numbers of elliptical and oval cells are present. The crowding out of the normal bone marrow elements and their precursors by leukemic tissue has been offered as a cause of thrombocytopenia, anemia and agranulocytosis. The mechanism of the anemia cannot be uniformly correlated however with the extent of bleeding or erythropoietic failure. Also the appearance of the bone marrow with regard to the extent of leukemic invasion is often unrelated to the degree of anemia.

The pathogenesis of the anemia is now viewed more as a dynamic process than a crowding out of erythroid elements.<sup>24</sup> It has been demonstrated by cell tagging techniques<sup>25, 26</sup> that the anemia of chronic leukemia is due in part to a decreased survival of erythrocytes perhaps caused by an intrinsic corpuscular defect. A similar increased hemolysis is also an important factor in advanced nonlymphomatous neoplastic disease.<sup>2</sup> When red cell production and red cell destruction were measured simultaneously in patients with leukemia, no single mechanism could be established for the causation of the anemia in every case. There was extreme variability between the degree of red cell survival (normal or short) and adequate or inadequate compensatory red cell production. Multiple factors therefore contribute to the anemia.

Illor, fatigability, dyspnea on exertion and listlessness are attributable to

the anemia. The increased basal metabolic rate in patients with acute leukemia is responsible for these symptoms as well as for anorexia, weight loss, and irregular fever.

*Relationship of Hyperleukocytosis to Severity of the Disease* The patients who present themselves with a marked leukocytosis at the onset are much more severely affected and the disease may run a more fulminant course than in those with leukopenia. When the disease has become stabilized, whether spontaneously or by therapy, and the cells are reduced in numbers, the clinical course is not significantly different from that of patients with a moderate leukocytosis or a leukopenia.

*Infections and Fever* The increased susceptibility of the patient to infection is attributable to the absence of granulocytes in the peripheral blood. It has been shown also that in persons with acute leukemia phagocytosis of *Brucella* organisms by mature neutrophils was not impaired<sup>83</sup> and that the absolute mature neutrophil count when infection develops varies from patient to patient. It is remarkable, however, that children can go for long periods with an agranulocytic blood picture free from infection without the continuous use of antibiotics. Other factors therefore must account for the development of bacterial infections in patients with acute leukemia. The bouts of fever, often with hyperpyrexia, usually are ascribed to infection, but the source is not always discernible. The predominance of negative blood cultures in our experience is probably the result of the immediate use of antibiotic and antimicrobial therapy with the onset of fever. Hemolytic *Staphylococcus aureus*, *Escherichia coli*, streptococci, and monilial infections have been the most frequent offenders in our series. In other reported series *Bacillus proteus* is also included.<sup>8</sup>

Infection contributes to the hemorrhagic tendency, and its control often coincides with subsidence of the bleeding. Localized infection in soft tissues leads to necrotic lesions requiring persistent and assiduous treatment but frequently remaining unhealed. Although usually confined to the oral cavity, monilial infections are particularly troublesome and may become widespread. In one of our patients moniliasis extended to the blood vessels of kidneys and spleen, producing extensive infarcts. Despite conflicting experimental data, there is suggestive evidence that the increased incidence of infection and tendency for this complication from ordinarily minor lesions and from organisms of low virulence seen in patients receiving the steroid hormones may be due to a failure of the tissues involved to localize the infection in a normal fashion.<sup>8</sup>

In a recent series<sup>84</sup> of ninety-two febrile episodes encountered in patients with acute leukemia, fifty-nine appeared to be related to infection. In the remaining thirty-three episodes, bacterial, viral, or fungal infections could not be incriminated as a cause for fever. In this group the most common clinical illnesses associated with the proved infections were pharyngitis, pyelonephritis, and septicemia. The most frequently encountered organisms were *Escherichia coli*, *Micrococcus pyogenes* var. *aureus*, coagulase positive, and *Pseudomonas aeruginosa*. The cause for persistent fever in the patients who had no demonstrable infection remained obscure. Penicillin, streptomycin, and tetracycline were the common drugs employed.

**Gastrointestinal Disturbances** The vomiting of blood may be caused by persistent oozing from the nasopharyngeal area or from ulcerations in the esophagus and stomach. Severe abdominal pain constitutes one of the most distressing symptoms and the source may not be obvious even on postmortem examination. The small punctate hemorrhages in the gastric mucosa, submucosal hemorrhage, enlarged Peyer's patches, infiltration of the mesentery of the small bowel and marked enlargement of the abdominal lymph nodes could account for the abdominal pain. For children under treatment with ACTH and steroid hormones, gastrointestinal ulceration and perforation are rarely found by the roentgenography or examination of the tissues, but these should not be overlooked. Necrotic ulcerative lesions of the lower rectum are associated with severe and persistent agranulocytosis as well as with monilial infection.

**Specific Organ Changes** The enlargement of superficial lymph nodes may be marked, or they may be of normal size.

The liver and spleen are very moderately enlarged and only occasionally reach enormous proportions, usually toward the end of the disease. The size of the spleen fluctuates spontaneously or with treatment; the liver remains more resistant to change. In patients with chronic myeloid leukemia the spleen shows progressive enlargement.

Rarely, the lacrimal and salivary glands in children may be involved in a symmetrical painless enlargement designated as the Mikulicz syndrome.<sup>44</sup>

**Pleural effusion or pneumonia or both** may be prominent during the disease. In one patient with eosinophilic leukemia confirmed by postmortem examination, persistent cough and fever were associated with extensive infiltration of both sides of the chest.

The lungs<sup>60a</sup> and kidneys<sup>6</sup> are most frequently involved, but the degree of infiltration of these and other organs is not necessarily correlated with the extent of their dysfunction. The kidneys frequently are markedly enlarged by the extensive parenchymal infiltration which is difficult to eradicate. Gross hematuria is common, but in some patients it is confined to the terminal phase, whereas in others it appears at the onset. Uremia is usually associated with very large kidneys. The increase in blood and urinary excretion of uric acid in patients with leukemia<sup>17</sup> results from the disintegration of large numbers of primitive cells with excessive catabolism of nuclear protein. It has been suggested that this factor coupled with the effects of infiltration may seriously interfere with kidney function. It has been stressed that the use of potent chemotherapeutic agents is capable of producing rapid and extensive destruction of blast cells with injurious effects on the kidney. The impairment of kidney function, however, is not a universal occurrence.

In a study conducted in a group of thirty-five children with leukemia, it was concluded that there was no evident impairment of renal function during the course of leukemia despite the presence of gross and microscopic infiltration of the kidney parenchyma at postmortem examination.<sup>36</sup> On the other hand, it has been stressed that impairment of renal function with delayed excretion markedly increases the blood level, and antileukemic drugs such as amethopterin produce severe toxicity.<sup>17</sup> These discordant opinions emphasize the need for individualiz-

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ing cases of acute leukemia in children especially with respect to the susceptibility to drug administration

The testes are infrequently involved in patients with acute leukemia. When affected they show progressive enlargement with nodular formation due to localized infiltration with leukemic cells.

The central nervous system is frequently involved in its many component structures by hemorrhage, thrombosis and infiltration by leukemic cells.<sup>6</sup> Neurologic signs and symptoms were present in 20 to 25 per cent of the patients in one series. Hemorrhage into the brain sufficiently extensive to be the immediate cause of death occurred in approximately 29 per cent.<sup>28</sup> Subarachnoid hemorrhage is also to be included in nervous system complications.

Exophthalmos due to retrobulbar hemorrhage or infiltration of the bones about the orbit is to be differentiated from chloroma which it closely resembles. Retinal hemorrhages are common and occasionally extensive and may be associated with impaired vision or actual blindness. Although these hemorrhages are ordinarily a valuable sign of intracranial bleeding they may be found in leukemic patients without other signs of nervous system involvement.<sup>28</sup>

The cranial nerves especially the sixth and seventh may be infiltrated and involvement is diagnosed by specific neurologic signs and symptoms.<sup>6</sup>

Increased intracranial pressure is a serious prognostic sign and is due to leukemic infiltration primarily of the meninges and to a lesser extent of the cerebral parenchyma. It may be asymptomatic but is frequently accompanied by severe headache, vomiting, meningismus, papilledema, drowsiness and coma. The cerebrospinal fluid is clear under increased pressure, the protein and cell count are elevated and microscopic examination reveals blast forms and a low or normal concentration of sugar. Roentgenograms of the skulls of the younger children show separation of the sutures. It has been suggested<sup>24</sup> that there has been a greater incidence of this complication in patients treated with the hormones and chemotherapy than in previously untreated patients. According to this premise the brain is attacked later than are the other organs so that prolonged survival permits the eventual involvement of the central nervous system. There is the possibility also that such agents as 6-mercaptopurine<sup>4</sup> and amethopterin cross the blood-brain barrier in greatly reduced amounts even though the disease is under control elsewhere. The latter feature should be emphasized namely that intracranial complications frequently occur while the peripheral blood and bone marrow are in a complete hematologic remission.

Leukemic meningitis occasionally simulates the bacterial form with cloudy fluid and decreased sugar content but examination of the cells reveals its leukemic etiology.<sup>4</sup>

**Differential Diagnosis.** Usually leukemia in childhood is diagnosed without difficulty after examination of the peripheral blood and bone marrow. In equivocal cases leukemia must be differentiated from disorders in which overlapping occurs in some aspect of the hematologic picture. The most important feature of the diagnosis of childhood leukemia is the critical evaluation of the clinical picture so that leukemia is suspected and the peripheral blood and bone marrow are examined.



**Normal Leukocytic Changes in Infancy and Childhood** Normal leukocytic changes in infancy and childhood may simulate leukemia. The lymphocytic increase in the first years of childhood must be kept in mind so that unnecessary significance is not attached to conditions in which these cells predominate. A mistaken diagnosis of leukemia can be made in young children because of a moderate elevation in the number of white blood cells, a preponderance of lymphocytes, the presence of small or moderately sized cervical nodes and a palpable spleen. In these children the white count is at the upper limit of normal as is the lymphocyte count. Furthermore, the finding of enlarged cervical lymph nodes and careful search of many smears fail to reveal blast cells. The cervical nodes are enlarged from repeated tonsillar infections and a palpable spleen with a smooth edge is not an uncommon finding in normal young children.

**Idiopathic Thrombocytopenic Purpura** Idiopathic thrombocytopenic purpura may be suggested by the appearance of petechiae, purpura and hemorrhagic manifestations. However, bone marrow aspiration reveals a conspicuous increase of megakaryocytes, whereas they are suppressed in patients with leukemia. In patients with thrombocytopenic purpura severe anemia is absent and the marrow reveals normal granulopoiesis and erythropoiesis in contrast to the anemia and infiltration by immature cells in patients with leukemia.

**Reticuloendotheliosis** In reticuloendotheliosis in infants not associated with characteristic skin lesions, sudden hematemesis and melena may occur with a diminution in the number of platelets. Bizarre reticuloendothelial cells occasionally may be identified in the bone marrow and skin scrapings<sup>69</sup> as compared with the marked invasion of both the peripheral blood and marrow by abnormal cells in patients with leukemia.

**Aplastic Anemia** The most frequent pancytopenic condition to be confused with leukemia is aplastic anemia. In patients with aplastic anemia the bone marrow shows a progressive decrease in the myeloid elements, nucleated red cells and megakaryocytes. In leukemia, except in the hypoplastic stage induced by chemotherapy or the infrequent spontaneous remission, the bone marrow and peripheral blood are infiltrated by immature cells.

**Neuroblastoma** The bone marrow in patients with neuroblastoma of the type referred to as sympathicoblastoma is sometimes sufficiently infiltrated by clusters of undifferentiated cells to suggest leukemia.<sup>70</sup> The identification of pseudorosettes with cells arranged about a central mass of fibrils in patients with neuroblastoma separates the two conditions morphologically. Also the peripheral blood of patients with neuroblastoma is free from abnormal cells. Other clinical features such as the presence of an abnormal mass with displacement of the kidney and focal areas of calcification are indicative of neuroblastoma. The roentgenogram cannot be employed for differentiation since leukemia of the skeleton closely resembles metastatic sympathicoblastoma.<sup>43</sup>

**Infectious Mononucleosis** Infectious mononucleosis can be differentiated by the absence of significant anemia, thrombocytopenia and close examination of the blood smear. Lymphadenopathy and splenomegaly may be slight to moderate, similar to early cases of acute leukemia. Sore throat may be present in patients

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count, and absence of splenomegaly in patients with the leukemoid reaction are contrasted with the severe anemia thrombocytopenia and enlarged spleen in those with leukemia. In fulminating cases of congenital leukemia the differentiation is often made only at postmortem examination when tissue infiltration with white blood cell precursors reveals the leukemic nature of the disease. In the patient with acute leukemia also there is a gap (hiatus leukemicus) between the myeloblast, lymphoblast or promyelocyte and the polymorphonuclear neutrophil which is not present in the patient with a leukemoid reaction.

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The serum of patients with infectious mononucleosis usually has a high titer of agglutinins for sheep cells. The elevated heterophil antibody titer encountered occasionally in patients with leukemia and other neoplastic diseases can be shown to be normal Forssman antibodies by means of an absorption test (complete absorption by guinea pig kidney).<sup>61</sup>

The atypical mononuclear cell in patients with infectious mononucleosis presents morphologic features distinct from those of the leukemic cell. Although the occasional Downey type III cells may resemble the lymphoblast, the appearance of significant numbers of characteristic atypical cells points to a diagnosis of infectious mononucleosis. In doubtful cases bone marrow aspiration will exclude leukemia, but this is rarely necessary.

*Acute Infectious Lymphocytosis* Acute infectious lymphocytosis occurs sporadically as well as in epidemic form and is characterized by a hyperleukocytosis due to an increase in normal small lymphocytes. The mildness of the clinical course, the absence of anemia, thrombocytopenia, and the lack of enlargement of spleen and lymph nodes serve to distinguish this condition from acute leukemia. Bone marrow examination fails to reveal the characteristic cell pattern found in patients with leukemia. The blood picture of patients with acute infectious lymphocytosis, however, may closely resemble that of those with chronic lymphocytic leukemia by the extreme elevation of white cells and the preponderance of small mature lymphocytes. The age incidence of the two diseases is very different, since chronic lymphocytic leukemia is a disease of older persons, rarely seen in those under 40 years of age.

*Rheumatic Fever and Rheumatoid Arthritis* Leukemic patients with low grade fever, slight anemia, leukopenia, cardiac murmur, and migratory bone and joint pain simulate the clinical and hematologic pictures in patients with rheumatic fever and rheumatoid arthritis.<sup>1, 62</sup> The diagnosis of leukemia is further obscured for a time at least by the absence of enlarged lymph nodes and spleen and of a clear cut blood picture. The presence of polyarthritis with the associated signs of local inflammation provides additional evidence of the possibility of rheumatic disease.<sup>63</sup> Suspicion as to the true nature of the disease should be aroused by the failure of the patient to respond to salicylates and by a favorable response to transfusion. Destructive changes of the skeleton observed by roentgenography exclude rheumatic fever and direct attention to leukemia as the underlying disease. Close examination of the blood smear, despite the paucity of white cells, usually reveals an occasional cell which can be identified as being leukemic in origin. Bone marrow aspiration subsequently confirms the diagnosis of acute leukemia.

*Leukemoid Reactions* The differentiation between leukemia and a leukemoid reaction may be difficult with respect to congenital leukemia and sepsis in the newborn infant and cases of chronic myelogenous leukemia in later childhood. Confusion exists particularly when the blood and bone marrow show increased numbers of myelocytes and transitional forms including metamyelocytes and segmented and nonsegmented polynuclear cells. The milder anemia, normal platelet

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in the form of transfusions of blood derivatives and antibiotics are directed respectively toward correction of the anemia and bleeding tendencies and treatment of infection.

**Chemotherapeutic Agents—Antimetabolites** The antimetabolites include the folic acid antagonists Aminopterin and amethopterin and the purine antagonist ■ mercaptopurine.

**Principle of the antagonist (analogues antimetabolites)** It has been well established that structurally related compounds may be biologically antagonistic. Antimetabolites in use in the treatment of leukemia are compounds whose chemical structure differs only slightly from a vitamin, an amino acid or a precursor of nucleic acid. These substances are so closely related chemically that they replace the metabolite in its particular enzyme system. Thus folic acid analogues combine with folic acid systems because of their similar chemical structure. In view of the structure not being the same as that of the metabolite, the reaction ceases and the enzyme system and in this case the utilization of folic acid is blocked.<sup>36,37</sup> For example, synthetic analogues of many vitamins which inhibit the growth of organisms have been prepared. Woods<sup>3</sup> originally observed that the bacteriostatic action of sulfanilamide was reversed by the chemically related para aminobenzoic acid. These observations led also to the concept that the analogues exhibit their effects by producing signs of deficiency of the metabolite which they resemble structurally.

The advent of potent antimetabolites which affect the synthesis of nucleic acid has focused considerable interest on its structure. The nucleic acids are composed of relatively simple primary units: phosphate, the sugars ribose or deoxyribose, the purines and the pyrimidines. The building materials involved in purine synthesis are glycine, carbon dioxide, ammonia and certain one carbon compounds such as formate. Folic acid is quite generally involved in one carbon unit metabolism and it appears to be specifically concerned with the synthesis of the purines. It is assumed that by virtue of this fact the antifolic acid compounds exert their growth inhibitory action. Thus nucleic acid synthesis might be interfered with by the use of folic acid antagonists (to block one carbon fragment incorporation) or by the use of purine or pyrimidine analogues (to block interconversion reactions and incorporation).

**Folic acid antagonists** Folic acid (PGA) a vitamin which occurs in nature and which is also synthesized consists of pteridine, para aminobenzoic acid and glutamic acid. Since it is essential for the growth of erythroid and myeloid cells of the bone marrow, megaloblastosis results when it is deficient. In addition to its specific action upon megaloblastic marrow, folic acid exerts many other nutritional effects and participates in specific metabolic functions frequently in conjunction with vitamin B<sub>12</sub>. Of basic importance is the fact that folic acid is essential for the growth of certain types of leukemic cells and with its derivatives plays an essential role in the biosynthesis of nucleic acid. Citrovorum factor (folinic acid, Leucovorin) is a derivative of and probably the active form of folic acid. Conversion of folic acid to the citrovorum factor is enhanced *in vivo* and *in vitro* by ascorbic acid.

Certain compounds biologically antagonistic to folic acid have the property

## Leukemia—Treatment

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The aim of therapy in patients with leukemia is the eradication of the leukemic process in the blood and tissues and the restoration of hematopoiesis to normal. No curative agent or method has as yet been discovered which permanently controls the disease. Although it is invariably fatal, substantial gains have been made in achieving remission and in prolonging life, especially in patients with acute leukemia of childhood.

**Treatment.** The principal therapeutic agents in current use in childhood leukemia are as follows:

1. Chemotherapeutic agents
  - A. Antimetabolites—inhibitors of cellular metabolism (probably at nucleic acid level)
    - (1) Folic acid antagonists
      - (a) Aminopterin
      - (b) Amethopterin (Methotrexate)
    - (2) Purine antagonist
      - (a) 6-Mercaptopurine (Purinethol)
  - B. Cytotoxic agents
    - A. Radiation therapy
    - B. Busulfan (Myleran)
2. Hormones
  - A. ACTH
  - B. Cortisone, hydrocortisone, prednisone, prednisolone
  - C. Triamcinolone, methylprednisolone, dexamethasone
3. Supportive treatment
  - A. Transfusions
  - B. Antibiotics

Skilful management depends upon the judicious selection of these agents for administration alone or in combination. Antileukemic therapy may be classified as specific and nonspecific. In the specific group reliance is placed mainly upon ACTH and the adrenocortical steroids, the folic acid antagonist methopterin (Methotrexate) and the purine antagonist 6-mercaptopurine, and to a lesser extent urethan, Myleran, and x-ray therapy.\* Supportive or nonspecific therapy

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\* Still under trial and of a lesser value than those mentioned are analogues of 6-mercaptopurine such as 6-thioguanine and 6-chloropurine; a aserine, an analogue of serine which is used either alone or in combination with 6-mercaptopurine; and demecolcin, a colchicine derivative.

of relapse which may precede clinical and peripheral blood changes. Unless the number of leukemic cells in the bone marrow is very low, relapses can occur quite rapidly. Occasionally enlargement of the spleen heralds a relapse. Repeated remissions may be obtained with amethopterin but each successive one is usually of shorter duration and more difficult to produce. It is of interest that children can be maintained on amethopterin for prolonged periods in relatively good health with the bone marrow and blood showing partial relapse.

**TOXIC EFFECTS** The initial symptoms of toxicity are loss of appetite and abdominal pains. The areas involved are the buccal mucous membrane, gastrointestinal tract and bone marrow. Small shallow ulcers which are painful and which interfere with feedings may appear on the buccal mucosa of the lips and the cheeks and on the tongue. At this point the dosage should be reduced or the drug discontinued otherwise serious ulceration of the gastrointestinal canal with massive hemorrhages develops. Erythematous and hemorrhagic rashes of the scalp, face, neck and trunk, nausea, anorexia, diarrhea, epistaxis and alopecia are other signs of toxicity. These manifestations including bone marrow hypoplasia or aplasia usually ascribed to toxic effects of the drug may represent part of the leukemic process itself.

A megaloblastic transformation of the bone marrow and peripheral macrocytosis represent true folic acid deficiency. The bone marrow may show varying degrees of injury with respect to the red and white blood cell elements and the megakaryocytes. Ultimately an aplastic bone marrow results with pancytopenia and bleeding unless the drug is withdrawn. With prolonged administration reversibility is more difficult to obtain. The simultaneous administration of ACTH or the steroid hormones with reduced dosages of amethopterin may be effective in controlling bleeding and in stimulating the aplastic bone marrow after the amethopterin has destroyed the blast cells. Although folic acid prevents the toxic effects of the folic acid antagonists it also interferes with their antileukemic action.<sup>3</sup> Nevertheless with the development of severe toxicity the drug should be withdrawn and Leucovorin may be administered intramuscularly (3 mg dosage). The value of Leucovorin is open to question once significant toxic effects have appeared.

**Purine antagonist—6 mercaptopurine (Purinethol)** This analogue of the nucleic acid constituent adenine and the physiologic purine base hypoxanthine, 6 mercaptopurine<sup>18</sup> has been found particularly effective in the treatment of acute leukemia in children. Its activity in leukemias which are resistant to the antifols is indicative of an antipurine rather than an antifolic acid mode of action. The drug interferes with the incorporation of purine into nucleic acid whereas the folic acid antagonists interfere with the synthesis of purines and pyrimidines involved in nucleic acid formation.

**DOSEAGE** The usual initial dose of 6-mercaptopurine is 25 mg per kilogram of body weight to the nearest 25 mg given once daily. The approximate dose is 50 mg in an average 5-year old child and in children of all ages the range is from 25 to 150 mg daily. The drug has a specific leukopenic effect which is manifested in seven to ten days after administration although maximal benefits require three to eight weeks of treatment. As with amethopterin 6-mercaptopurine may be

of inhibiting cell growth. The folic acid antagonists that have been most widely used are Aminopterin and amethopterin (Methotrexate). Both involve a change in the folic acid molecule by the addition of an amino group in the 4 position of the pteridine ring. The administration of either of these antagonists in controlled doses prevents the utilization of folic acid. Normal as well as leukemic tissues are affected by these agents but the development of rapidly growing immature cells is impaired more than that of the mature slowly growing cells. The body therefore is placed in a state of folic acid deficiency insufficient to injure normal cells.<sup>3</sup>

The analogues Aminopterin (4 aminopteroylglutamic acid) and amethopterin (4 amino N<sup>10</sup> methylpteroylglutamic acid) appear to exert their antimetabolic effect by blocking the conversion of folic acid to citrovorum factor itself. These folic acid derivatives enter into the synthesis of purines and pyrimidines and in blocking the activity of purines and pyrimidines nucleic acid formation is reduced and cell growth is retarded.

In many instances it seems likely that the leukemic cells rapidly acquire resistance to the folic acid and other antagonists. Thus experience has shown that eventually all patients ultimately become resistant to these compounds but the mechanism by which this is accomplished is not definitely established.<sup>10</sup>

**AMINOPTERIN** The chemotherapeutic agent Aminopterin was among the first of the analogues of folic acid to be employed successfully in the treatment of patients with acute leukemia.<sup>8, 7</sup> It is given in amounts of 0.5 to 1 mg daily orally or intramuscularly (at all pediatric age periods). Although substantial improvement and complete remissions have been obtained with this drug clinical experiences have demonstrated the superiority of amethopterin. The latter possesses a much wider margin of safety and therefore is less prone to produce toxicity from the dosages used than is Aminopterin.

**AMETHOPTERIN (METHOTREXATE)** The dosage of amethopterin ranges from 1.25 to 5 mg given orally once daily. The usual dose is 1.25 mg for very small children and 2.5 mg daily for children over 2 years of age. At this dosage treatment can be carried out for prolonged periods without toxic effects. In some patients 5 mg is required to obtain a remission but at this level evidences of toxicity frequently appear. The drug is rapidly absorbed and constant supervision is necessary to avoid serious complications. Intermittent therapy is sometimes useful in children particularly those susceptible to toxic reactions. The regimen of ten days with and four days without the drug has proved of value in our hands.

A remission following the administration of folic acid antagonists generally takes three to eight weeks although satisfactory responses have been obtained within ten days to two weeks. Remissions with this drug can be maintained for periods of several months to a year and longer by skillful management by either continuous or intermittent therapy. Good clinical and hematologic remissions may be expected in 30 to 50 per cent of patients.<sup>2</sup> Following a remission therapy is usually continued on a dosage adjusted to maintain a normal blood and bone marrow status without signs of toxicity. During treatment the bone marrow should be examined at two to four week intervals to detect early signs

of relapse which may precede clinical and peripheral blood changes. Unless the number of leukemic cells in the bone marrow is very low relapses can occur quite rapidly. Occasionally enlargement of the spleen heralds a relapse. Repeated remissions may be obtained with amethopterin but each successive one is usually of shorter duration and more difficult to produce. It is of interest that children can be maintained on amethopterin for prolonged periods in relatively good health with the bone marrow and blood showing partial relapse.

**TOXIC EFFECTS** The initial symptoms of toxicity are loss of appetite and abdominal pains. The areas involved are the buccal mucous membrane, gastrointestinal tract and bone marrow. Small shallow ulcers which are painful and which interfere with feedings may appear on the buccal mucosa of the lips and the cheeks and on the tongue. At this point the dosage should be reduced or the drug discontinued otherwise serious ulceration of the gastrointestinal canal with massive hemorrhages develops. Erythematous and hemorrhagic rashes of the scalp, face, neck and trunk, nausea, anorexia, diarrhea, epistaxis and alopecia are other signs of toxicity. These manifestations including bone marrow hypoplasia or aplasia usually ascribed to toxic effects of the drug may represent part of the leukemic process itself.

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**Purine antagonist—6 mercaptopurine (Purimethol)** This analogue of the nucleic acid constituent adenine and the physiologic purine base hypoxanthine. 6 mercaptopurine<sup>18</sup> has been found particularly effective in the treatment of acute leukemia in children. Its activity in leukemias which are resistant to the antifolates is indicative of an antipurine rather than an antifolic acid mode of action. The drug interferes with the incorporation of purine into nucleic acid whereas the folic acid antagonists interfere with the synthesis of purines and pyrimidines involved in nucleic acid formation.

**dosage** The usual initial dose of 6 mercaptopurine is 2.5 mg per kilogram of body weight to the nearest 25 mg given once daily. The approximate dose is 50 mg in an average 5-year-old child and in children of all ages the range is from 25 to 150 mg daily. The drug has a specific leukopenic effect which is manifested in seven to ten days after administration although maximal benefits require three to eight weeks of treatment. As with amethopterin 6 mercaptopurine may be

increased above the stated dosage when the response is inadequate especially in the later stages of the disease

The drug is discontinued temporarily at the first evidence of a precipitous drop in the white blood cell count especially in patients with marked leukocytosis since a continued drop often follows the cessation of therapy. When the leukocyte count rises or becomes stabilized for several days with counts ranging from 1 000 to 1 500 white cells per cubic millimeter treatment is reinstituted. The same program is followed with elevated but more moderate initial white blood cell counts. Periodic bone marrow aspiration is useful in determining to what extent the peripheral leukopenia is associated with leukemic infiltration. The proliferation of the megaloblastic series characteristically seen with folic acid antagonists does not occur with 6 mercaptopurine. If the bone marrow is still heavily involved treatment can be resumed provided the blood count does not fall below 1 000 white blood cells per cubic millimeter. From this level the white blood cell count usually rises again within a few days. A rapid decrease in the white blood cells in patients with hyperleukocytosis occasionally results in hyperuricemia and impaired renal function.

A suitable maintenance dose should be established depending upon the character of the response in the initial treatment. As with amethopterin 6 mercaptopurine may be given continuously or intermittently following a remission. Relapses usually occur within four to six weeks after treatment is stopped. Good clinical and hematologic remissions were observed in 47 per cent of patients in one series of eighty seven children.<sup>3</sup> The drug produces remissions in children with acute leukemia who have been previously untreated and in those in whom the disease has become resistant to amethopterin and the steroid hormones. Clinical improvement with reduction in size of the liver spleen and lymph nodes accompanies the return to normal of the bone marrow and peripheral blood. 6 Mercaptopurine is rapidly metabolized disappearing from the plasma almost completely in twenty four hours. Only in a dosage higher than that usually prescribed does the drug pass the blood brain barrier in appreciable amounts.<sup>12</sup>

**TOXIC EFFECTS** The chief toxic manifestations are hematologic with marked leukopenia and bone marrow aplasia. The drug is less toxic to the mucosa of the gastrointestinal tract than are the folic acid antagonists. Mouth lesions nausea vomiting anorexia and mild diarrhea occur exceptionally.

**6 Mercaptopurine combined with azaserine** The combination of 6 mercaptopurine and azaserine has been subjected to clinical trial in the treatment of patients with acute leukemia following the demonstration of its synergistic therapeutic effect in experimental animals. Azaserine an analogue of serine which also blocks nucleic acid synthesis is given orally in a dosage of approximately 25 mg per kilogram of body weight. Ulceration of the tongue and buccal mucosa is an indication for temporary withdrawal of this drug alone. It is later resumed at a lower dosage of 125 mg per kilogram of body weight together with the full dose of 6 mercaptopurine. In one series complete remissions were obtained with an average duration of six months thus delaying the resistance to 6 mercaptopurine alone on the average of about two months. In another and

more extensive study,<sup>1,4</sup> the duration of the remissions obtained with the combined therapy was not significantly longer than when 6-mercaptopurine was used alone.

**Cytotoxic Agents** The principle cytotoxic agents used in the treatment of childhood leukemia are radiation therapy and the drugs urethan and busulfan (Myleran).

**Radiation therapy** Roentgen ray irradiation finds a place in the treatment of acute leukemia in childhood. It is effective in relieving pressure symptoms and pain due to enlargement of infiltrated organs and localized leukemic masses. It is an excellent adjuvant in relieving severe headache in patients with acute leukemia and in intractable bone and joint pain and disability in those with any type of leukemia, especially in the infrequent chronic myelocytic form in children. In patients with chronic myelocytic leukemia, especially, it reduces the leukocytic count and the number of immature forms. The technique of treatment is highly specialized and requires a consideration of the advantages of local versus generalized irradiation. As with the specific drugs, treatment needs to be carefully controlled by regular hematologic examinations so as to avoid pancytopenia and aplastic bone marrow.

**Busulfan (Myleran)**—*use in patients with chronic myelocytic leukemia* The search for a less toxic analogue of nitrogen mustard led to the discovery of a sulfonic acid ester, busulfan. This drug has been found to be invaluable in the treatment of chronic myelocytic leukemia,<sup>11,19</sup> and of little or no value in other types of leukemia. It has largely replaced irradiation by x ray and the mitotic inhibitors urethan (ethyl carbamate) and demecolcin (a derivative of colchicine) previously used in treatment of this type of leukemia.

**MODE OF ACTION** Busulfan is myelotoxic and depresses normal and abnormal myeloid tissue. It tends to act selectively on granulocytes, but in large doses platelets, red cells, and lymphocytes are also depressed. Reduction in the leukocyte count is the prominent feature and may be achieved in ten to fourteen days or delayed for three or four weeks. The remission is accompanied by the correction of the anemia, a reduction or even a disappearance of the immature myeloid cells from the blood, a decrease in the cellularity of the bone marrow, a diminution in the size of the liver and spleen, and pronounced subjective improvement. Large daily doses may produce thrombocytopenia and serious bone marrow depression. Toxic side effects are confined to the bone marrow, and little if any other discomfort is encountered. Prolonged remissions lasting six to twelve months have been reported in adults.

**DOSAGE** Busulfan is available in 2 mg tablets. The usual dosage is 4 mg daily until a maximum hematologic and clinical improvement is obtained. Administration of the drug is continued until the white blood cell count drops to approximately 10,000 per cubic millimeter. Occasionally 6 mg per day is required to attain a remission. However, the amount given must be individualized, especially in children, for a precipitous decrease in the leukocyte count may develop necessitating withdrawal of the drug until the white blood cell count is stabilized. The need for maintenance therapy is still debatable. A small dose adjusted to the particular patient may be given to prevent elevation or

further reduction in the white blood cell count. Some resume therapy when there is a substantial rise in the leukocyte count.<sup>34</sup>

Radioactive phosphorus ( $P^{32}$ ) and nitrogen mustard and its derivatives such as triethylene melamine (TEM) although effective in patients with chronic leukemias have not been found to be particularly valuable in those with acute leukemias.

**Hormones** The hormones used in the treatment of leukemia in childhood include ACTH, cortisone, hydrocortisone, prednisone, prednisolone, triamcinolone, methylprednisolone and dexamethasone.

**Hematologic response in normal subjects** The blood response to the administration of ACTH in human subjects with unimpaired adrenal function is well known—within four hours of the intramuscular injection of 25 mg. of ACTH, there is an increase in circulating neutrophils and a decrease in lymphocytes and eosinophils.<sup>35</sup> The involution of lymphoid tissue and dissolution of lymphocytes previously reported with the adrenocortical hormones provided a basis for clinical trial of these hormones in human beings with leukemia. The mechanisms by which neutrophilia, eosinopenia and lymphocytopenia are produced are not entirely understood nor has the mode of action in leukemia found a satisfactory explanation. The inhibition of incorporation of a structural analogue of thymidine ( $I^{131}$  labeled iodoxyuridine) into DNA by cortisone may implicate the latter directly or indirectly in nucleotide metabolism.<sup>36</sup>

**Role in treatment of leukemia** Corticotrophin (ACTH) and the adrenocortical steroids (cortisone, hydrocortisone, prednisone and prednisolone) have proved particularly effective in the treatment of acute leukemia in childhood. In contrast to the folic acid antagonists and 6 mercaptopurine which require three to eight weeks to produce a satisfactory response, these hormones act rapidly. When serious bleeding occurs and the patient's condition is otherwise precarious the hormones are the treatment of choice.

Rapid and dramatic improvement has been brought about by these hormones in critically ill patients within several days of treatment and complete remission has been noted in three to four weeks. Satisfactory clinical and hematologic remissions occur with the initial use of ACTH and the steroid hormones in 50 to 75 per cent of children with acute leukemia.<sup>3, 37, 38</sup> The response in patients with acute myeloblastic and monoblastic leukemia is much less favorable.

In the first weeks of successful hormone therapy there is a disappearance of fever and return of appetite, greater activity, a subsidence of bone and joint pain, a lessening of hemorrhagic manifestations and a regression of enlarged lymph nodes and spleen. Within one week the white blood cell count decreases, the blast cells decrease in the blood and bone marrow and the reticulocyte count rises. Platelets increase in number after the first week and hemoglobin levels rise. Bone marrow hypoplasia is not infrequently observed during the second week of treatment. Erythroblastic and granulocytic cells make their appearance and in many patients a complete remission is achieved by the third to fourth week of treatment. Remission is relatively short and in most patients extends from three weeks to three months but it occasionally extends from six to



nine months without further treatment. Successive remissions are less frequent, incomplete and of shorter duration.

**Types and dosage.** No definite dosage can be stated for hormonal therapy since the speed of response and signs of hypercorticism which limit its use are subject to individual variation. The following schedule serves as a guide in treatment and is based upon dosages in current use. In order to provide sustained hormonal effects the total daily dosage must be divided.

**ACTH.** ACTH is administered in 25 mg doses by continuous intravenous drip in a glucose infusion over an eight to twelve hour period. Rarely is more than one treatment given daily. Intramuscular injection of an aqueous preparation or preferably the gel of 60 to 80 units daily is given in two divided doses to children under 4 years of age and 80 to 120 units is given to older children.<sup>22</sup> ACTH is rapidly absorbed following intravenous or intramuscular injection.

**CORTISONE.** Cortisone is administered orally in a total daily dosage of 100 to 200 mg. It is given in divided doses at six hour intervals. It has been largely replaced by hydrocortisone.

**HYDROCORTISONE.** Hydrocortisone (compound F) can be given orally intravenously or intramuscularly depending upon the urgency of treatment. It produces maximal physiologic effects when given by continuous intravenous drip. Because its effects are terminated rapidly when intravenous administration is stopped it is necessary to supplement it with the oral or intramuscular preparation. The intravenous dosage is 100 mg daily. Total oral dosage is 60 to 80 mg in younger children and 80 to 120 mg in older children. Hydrocortisone is given at six hour intervals in four divided doses.

**PREDNISONE AND PREDNISOLONE.** Prednisone and prednisolone serve as initial treatment or to maintain the improvement instituted by ACTH or to replace the antimetabolites in the event of relapse. They possess the advantage of rapid onset of action and prompt therapeutic effects. These hormones are available in 5 mg tablets and are given at six hour intervals. In young children the tablets are crushed in stewed fruit or apple sauce.

These compounds are produced by subtle modification in chemical structure of cortisone and hydrocortisone respectively. Thus endowed with a potency three to five times greater than that of the parent compounds they possess relatively marked decreases in electrolyte side effects. In moderate dosage (approximately 1.5 mg per kilogram) therapy is not complicated by sodium retention and excessive potassium excretion which occur with the older corticosteroid agents. Although these precautions therefore do not apply to prednisone and prednisolone in average therapeutic doses hypopotassemia, sodium retention and elevated blood pressure may occur when massive or excessive amounts are given over prolonged periods and appropriate measures must be taken to obviate these side effects.

Prednisone and prednisolone are administered orally in doses of 15 to 20 mg daily in children under 2 years of age and in doses of 20 to 60 mg daily in older

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Hydrocortisone succinate (Solu-Cortef, Upjohn) which contains 100 mg. of hydrocortisone in each vial is particularly suitable for intravenous use especially in emergency situations when high levels of hydrocortisone are needed immediately.

children. The average effective dose in children over 2 years old totals 40 to 60 mg daily in divided dosage with the higher level in the first two to three days.

These steroids are gradually withdrawn when the bone marrow returns to normal. Following cessation of therapy the bone marrow is re-examined at two- to four-week intervals. In our experience maintenance therapy with small doses of prednisone given daily or intermittently has not prolonged the remission.

Under present trial are new synthetic corticosteroids as substitutes for prednisone and prednisolone. These are triamcinolone (Aristocort and Kenacort), methylprednisolone (Medrol)† and dexamethasone (Deronil and Decadron)‡. It is claimed that the newer compounds require smaller dosage and produce a lesser incidence of side effects. The 4 mg tablet of Aristocort, Kenacort, and Medrol corresponds to the 5 mg tablet of prednisone so that if trial is contemplated the dosage should be adjusted on the basis of four-fifths of Meticorten. One 0.75 mg tablet of Decadron or Deronil replaces 4 mg of methylprednisolone or triamcinolone or 5 mg of prednisone or prednisolone.

When a complete clinical and hematologic remission has been achieved no further specific therapy is given until a relapse is in evidence. Since subsequent remissions following the administration of the hormones are progressively shorter other chemotherapeutic agents are employed. In some patients amethopterin has been instituted following the remission with hormones.

Cross resistance between the hormones and folic acid antagonists or purine antagonists does not occur so that a patient who has become resistant to any of these agents may still be expected to respond to the others.

**Massive hormone therapy.** Remissions have been reported in adults and to a lesser extent, in children with acute leukemia of various types morphologically differentiated and undifferentiated following massive doses of prednisone and prednisolone. Dosages of 1 gm daily (occasionally higher) have been recommended until satisfactory improvement is obtained with a variable toxicity.<sup>16-18</sup> Since the results with amethopterin and 6-mercaptopurine and lower doses of steroids have been satisfactory in children the use of massive therapy may find a place in the terminally ill patient when all other possibilities and combinations of therapy have been exhausted.

**Toxic and side effects.** Serious toxic and side effects indicative of hypercorticism develop with prolonged and excessive administration. They consist of acneiform eruption, hirsutism and increased deposition of fat in various parts of the body giving the "moon face" and the "buffalo hump" effect on the back of the neck. Edema, osteoporosis, glycosuria and elevated blood sugar, a tendency toward infections and clinical, chemical and electrocardiographic evidences of hypokalemia represent other diverse effects. Encephalopathy and cerebral hemorrhage in patients with severe thrombocytopenia also necessitate a reduction or withdrawal of hormone therapy. Severe hypertension however is a rare complication in childhood.

**Adrenocortical steroids combined with amethopterin and 6-mercaptopurine.** Either amethopterin or 6-mercaptopurine may be combined with the steroids

Aristocort—Lederle; Kenacort—Squibb

†Medrol—Upjohn

‡Deronil—Schering; Decadron—Merck Sharp & Dohme

in the event of bleeding phenomena persistent hypoplastic marrow signs of nervous system involvement headache and anorexia Prednisone is given in a dosage of 20 to 40 mg depending upon the age of the patient

*Adjuvant treatment with the adrenocortical steroids* Patients treated with adrenocortical hormones are maintained on a low salt diet with additional potassium in the form of potassium chloride 0.3 gm given orally several times daily This caution applies particularly to patients being treated with ACTH cortisone and hydrocortisone With the latter also sodium and potassium determinations should be carried out initially and repeated only for clinical indications With this group of newer steroids (prednisone etc) sodium retention is not significant excessive potassium loss is easily controlled by having the patient take potassium chloride tablets or foods rich in potassium such as orange juice grapefruit or bananas

*Remissions—Criteria* Therapeutic responses are classified as either complete or partial depending upon the extent to which the bone marrow peripheral blood and organ involvement revert to normal In accordance with the recently established standards of the Leukemia Chemotherapy Cooperative Study Group A<sup>1</sup> a complete remission includes the following criteria In the bone marrow there is reduction in the number of blasts to less than 10 per cent with lymphocytes to less than 20 per cent and essentially normal appearing granulopoiesis erythropoiesis and thrombopoiesis In the peripheral blood there is return to and maintenance for more than one month of hemoglobin greater than 11 gm per 100 ml for children under 15 years of age or 10 gm for infants under 2 years of age granulocyte levels in excess of 1500 per cubic millimeter platelet counts greater than 100 000 per cubic millimeter and an absence of leukemic cells in the blood smear Clinically signs ascribable to leukemia should be absent Criteria of a partial remission and a relapse involve increasing clinical and hematologic evidences of leukemic infiltration

With satisfactory hematologic and clinical improvement the patient should return to complete health and activity appetite should be normal and no overt toxic effects of drug therapy should be manifested

*Detailed Program of Treatment* With the diagnosis of leukemia the following factors require consideration choice of chemotherapeutic agent in relation to type of leukemia choice of a drug with which to initiate treatment the need for blood transfusions and other supportive therapy and the question of hospitalization and of psychological factors especially with relation to the parents

*Choice of Chemotherapeutic Agent in Relation to Type of Leukemia* Experience has demonstrated that significant differences in the therapeutic effects of various chemotherapeutic agents depend upon the type of leukemia diagnosed Following are the chemotherapeutic agents of choice in the treatment of various types of leukemia

*Acute stem cell (lymphoblastic) leukemia*

- 1 ACTH
- 2 Adrenocortical steroids
- 3 Amethopterin (Methotrexate)
- 4 6-Mercaptopurine (Purinethol)

*Eosinophilic leukemia*

- 1 Adrenocortical steroids
- 2 ■ Mercaptopurine (Purinethol) (less effective)

*Acute myeloblastic promyelocytic and myelocytic leukemia and monocytic leukemia*

- 1 ■ Mercaptopurine (Purinethol)
- 2 Amethopterin (Methotrexate)
- 3 Adrenocortical steroids

*Chronic myelocytic leukemia*

- 1 Busulfan (Myleran)

The acute stem cell (lymphoblastic) leukemia responds to ACTH and the adrenocortical steroids amethopterin and 6 mercaptopurine. Eosinophilic leukemia responds principally to the adrenocortical steroids and less favorably to 6 mercaptopurine. Acute myeloblastic promyelocytic and myelocytic and monocytic leukemias respond poorly to the adrenocortical steroids and better to 6 mercaptopurine. Following relapse with 6 mercaptopurine amethopterin is used next and steroids are reserved when other agents fail. Chronic myelocytic leukemia responds to busulfan. Although these recommendations are more or less specific it is frequently necessary to deviate from them to introduce another drug when the response is greatly delayed. The antimetabolites may have to be pushed however to the point of inducing severe leukopenia before deciding on their ineffectiveness. To accomplish this the dosage may have to be increased with a close watch for toxicity. At present busulfan appears to be the drug of choice in the treatment of chronic myelocytic leukemia.

With the exception of ACTH and the adrenocortical steroids the anti-leukemic agents enumerated are capable of injuring the bone marrow. The fact that myelotoxicity responsible for bone marrow aplasia is also destructive to leukemic cells suggests that for an agent to be of value therapeutically it must also have myelotoxic properties.<sup>4</sup> This fact must be kept in mind in prolonged treatment with a chemotherapeutic agent and emphasizes the need for periodic bone marrow examinations.

Occasionally early in treatment the antimetabolites produce signs of toxicity of sufficient severity to warrant withdrawal of the drug. These signs consist of anorexia, listlessness, fever, extreme leukopenia and increased bleeding from the gums and other areas. In such an event the adrenocortical steroids and fresh whole blood are valuable in tiding the patient over the period until the specific therapy is reinstituted. It may be necessary in the sensitive patient to continue adrenocortical steroids in small dosage combined with the appropriate amount of amethopterin or 6 mercaptopurine.

From the response in normal subjects ACTH and the adrenocortical steroids appear to be contraindicated in patients with acute granulocytic leukemias. Although they possess the potentiality of producing exacerbations in patients with this type of leukemia they may be used to advantage at least temporarily in controlling hemorrhage and in restoring the well being of the patient.

*Choice of Drug With Which to Initiate Treatment* The principal chemotherapeutic agents in current use in acute leukemia in childhood are amethopterin, ■ mercaptopurine and ACTH and the adrenocortical steroids. The practice

is to prescribe each drug in turn as resistance develops. Except for the adrenocortical steroids the same drug with which a remission is achieved is continued in smaller dosage for maintenance therapy.

In treating with either amethopterin or 6 mercaptopurine the dosage is increased before shifting to the other class of antimetabolites. Combination therapy with any two or three of the agents is eventually resorted to in the later stages of the disease when there is no response to each separately since there is no cross resistance between them. But there is no evidence that the combination of these drugs proves any more effective at any stage of the disease than each given separately in influencing the length of the remission.

Amethopterin and 6 mercaptopurine produce longer remissions than the adrenocortical steroids but generally require three to eight weeks for a maximal response. With both drugs satisfactory responses occasionally have been observed within a week or ten days.

With ACTH and the adrenocortical steroids remissions occur within one to three weeks but are shorter than with the antimetabolites. Occasionally remissions of seven and eight months have been observed without therapy following the initial use of prednisone. Although each remission with the hormones becomes progressively shorter nevertheless they may be repeated following their initial use as a relapse develops. Following the administration of adrenocortical steroids the length of the remission in individual patients may be so prolonged that it is common practice not to institute another drug until evidences of a relapse are noted. An alternative plan infrequently adopted is to introduce amethopterin in full dosage just as soon as the initial remission with the adrenocortical hormones occurs. The latter program requires long term evaluation with respect to comfort to the patient and length of survival. The steroids are employed to control bleeding manifestations at any time during the disease regardless of the drug in current use.

In the gravely ill child with other than stem cell or undifferentiated leukemia usually the granulocytic and monocytic types ACTH and the hormones are nevertheless administered. These agents are supplemented by supportive treatment until the immediate emergency is over when the specific drug may be given.

With these guiding principles as a basis current practices in the treatment of the acutely ill child who is febrile and in whom hemorrhage is manifest has been to initiate therapy with ACTH and the adrenocortical steroids. The drug is also indicated in the occasional patient with acute stem cell or undifferentiated leukemia with a hyperleukocytosis who is not critically ill.

Since the white blood cell count frequently undergoes a precipitate drop with steroid treatment an alternate method in the relatively well child with marked elevation of leukocytes is to prescribe 6 mercaptopurine so as to avoid too rapid dissolution of white blood cells leading to hyperuricemia and kidney impairment. In our own experience such onward events have not occurred although cases of hyperleukocytosis are carefully controlled by blood urea acid and urea nitrogen determinations. In the majority of patients with leukemia the clinical condition is fairly good and the white blood cells range from a leukopenia to a moderate leukocytosis. The best results however depend upon early

*Eosinophilic leukemia*

- 1 Adrenocortical steroids
- 2 6 Mercaptopurine (Purimethol) (less effective)

*Acute myeloblastic promyelocytic and myelocytic leukemia and monocytic leukemia*

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once by platelet rich plasma also obtained by plastic bag technique and given continuously unless the hemoglobin is reintroduced. By assiduous application of this program of intensive and continuous therapy bleeding is usually controlled.

It should be remembered that patients receiving multiple transfusions of whole blood may occasionally develop thrombocytopenia due to the formation of antibodies against platelets.

Concentrated platelets in patients with severe bleeding have proved effective in producing temporary hemostasis<sup>19</sup> but in an emergency they are usually difficult to prepare. As has already been stated ACTH and cortisone have been shown to increase capillary resistance<sup>20</sup> so that these hormones as well as prednisone are useful adjuncts in the control of bleeding. It has been shown however that ecchymoses may occur in patients undergoing prolonged ACTH and steroid therapy.<sup>21,22</sup> The administration of ascorbic acid (200 to 300 mg daily) has therefore been recommended as supplementary treatment.<sup>23</sup>

Bleeding from accessible areas is occasionally controlled by the local application of thromboplastin Gelfoam or packs saturated with a mixture of thrombin and Adrenalin.

Unless there is associated hemorrhage transfusions are not required until hemoglobin levels decrease to 7 or 8 gm per 100 ml. The dosage of whole blood packed cells and plasma for the different age groups are listed in Chapter 7.

**Antibiotics.** Broad spectrum antibiotics are frequently given routinely when ever adrenocortical steroids are administered. Prolonged administration of ACTH and of the steroids is known to inhibit the inflammatory process with the result that serious infection can develop without the usual clinical signs and symptoms. Advocates of this policy also cite the fact that the leukemic patient is prone to develop infections as a result of the depletion of myeloid elements in the blood and bone marrow.

The difficulties with routine use of the antibiotics with the steroids and the antimetabolites are the possibility of eventual resistance to specific pathogenic organisms and the development of widespread and fulminating monilial infection. A more realistic approach is to administer antibiotics when infection is present. This is often manifested in the initial stages of the disease and at varying periods subsequently.

With severe and prolonged infection when sepsis is suspected the choice of an effective antibiotic depends upon bacteriologic methods. It is not always possible however to isolate the causative organism in order to determine its susceptibility to antibiotics by *in vitro* tests. The tetracyclines have been effective and are given orally in a dosage of 20 to 40 mg per kilogram of body weight. When bleeding is minimal injections can be given intramuscularly. Penicillin in combination with streptomycin is administered once or twice daily each in a total dosage of 300 000 to 600 000 units and 20 to 40 mg per kilogram of weight respectively. Chloramphenicol given orally in a dosage of 40 mg per kilogram often has been found singularly effective in infections refractory to other antibiotics despite its known toxic effect on bone marrow. It may be administered by drip intravenously in critically ill patients in a dosage of 100 mg per kilo

detection of the disease and control of its progress is vigorously as possible with antileukemic therapy

Since there is no urgency for a rapid control of the disease in the mild cases it is the custom to initiate therapy with the antimetabolites methotrexin or 6 mercaptopurine a low platelet count being no contraindication to their use. Experience has shown that either antimetabolite may be given initially to the patient with the milder type of acute stem cell leukemia. According to this plan ACTH and the adrenocortical steroids are kept in reserve for the periods when the antimetabolites can no longer control the disease.

It is impossible to set forth absolute criteria for the administration of anti-leukemic therapy except in the seriously ill child in whom ACTH and the adrenocortical steroid therapy is mandatory. The individual response is so varied with each class of compounds that judgment must be exercised in the replacement of one drug by another and in relation to dosage. Some clinicians prefer to postpone antileukemic therapy until the symptoms and clinical findings progress hoping thereby to conserve essential therapy and prolong the period of survival. Most commonly therapy is initiated in all patients with leukemia as soon as the diagnosis is established. In our clinic except in patients with the mildest form of the disease in whom antimetabolites are prescribed we have preferred to initiate treatment with prednisone. Since a remission is predictable with this agent in at least 70 per cent of patients with acute stem cell leukemia within three to four weeks of treatment against a more prolonged period for the antimetabolites the parents are given an opportunity to orient themselves with regard to the seriousness of the disease and subsequent management within the initial period of hospitalization.

**Supportive Therapy** Transfusions and antibiotics are the methods of supportive therapy chiefly relied upon in patients with leukemia.

**Transfusions** Blood is administered to combat anemia and to control hemorrhage. There is also evidence that the action of blood may not be confined solely to its capacity for raising hemoglobin but that it may also be responsible for an occasional remission. This rare phenomenon noted after ordinary transfusions has led to the hypothesis that normal blood contains an antileukemic substance. More directly it has been shown that fresh blood rather than stored blood was effective in producing a decline in the total number of leukocytes without however modifying the differential count.<sup>2</sup>

The hemoglobin value at which a transfusion is indicated varies with the associated signs and symptoms present in the patient. Although the requirements for raising hemoglobin levels to correct anemia can be met by the administration of packed cells whole blood is often more desirable because thrombocytopenia is commonly present especially during chemotherapy. Fresh whole blood obtained in siliconized equipment or platelet rich plasma derived from such a sample has proved remarkably effective in the control of hemorrhage.

For overwhelming and persistent hemorrhage from the nose mouth and gastrointestinal canal we have found that a routine of continuous treatment with whole blood and its products is beneficial. Fresh blood collected in a plastic bag is given until the hemoglobin level is normal. This is followed at



marrow material might conceivably generate normal blood elements. The results thus far have been uniformly unsuccessful although questionable transient improvement has been cited.<sup>1</sup> The collecting and storing of human marrow from living persons or cadavers for subsequent injection present formidable problems which are now in the process of solution. The successful transplantation of human bone marrow would offer long sought for help in the treatment of patients with aplastic anemia, leukemia, and disseminated neoplastic disease.

**Laboratory Determinations** In the patient who is hospitalized daily peripheral blood counts are important to determine the effect on the leukocyte count and leukemic cells. Platelet and reticulocyte counts may be done periodically. The bone marrow is examined at the outset and again at the end of the second and fourth weeks of initial treatment. Subsequently peripheral blood counts may be carried out at weekly or biweekly intervals. Bone marrow examinations are required if suspicious signs of relapse develop, if marked leukopenia is noted in the peripheral blood, or if the patient's condition deteriorates so that a change in therapy is contemplated.

Blood urea nitrogen and uric acid estimations are necessary in the event of a precipitous fall in the white blood cell count with signs of obstructive uremia.

**Treatment of Nervous System Involvement** Nervous system involvement has been noted with increasing frequency in children whose lives have been prolonged with chemotherapy.<sup>2-4</sup> Frequently neurologic symptoms occur while the peripheral blood and bone marrow are in remission. Vomiting, papilledema, separation of the cranial sutures, headache, stiff neck, and pleocytosis (lymphoblasts) point to leukemic infiltration of meningeal and nerve tissues. Irradiation to the brain or vertebral column to supplement chemotherapy has often been effective in relieving symptoms and in decreasing the cells in the spinal fluid. ACTH or steroid hormones alone or in combination with amethopterin or 6-mercaptopurine in the usual or increased dosage have also been useful.

Because of its poor penetration into the cerebrospinal fluid, amethopterin should be given intrathecally.<sup>2,3</sup> The dose is either 0.25 mg per kilogram of body weight every second to third day or 0.5 mg per kilogram every fourth to fifth day with an additional dose after the spinal cell count has returned to normal.<sup>1</sup> The dose is given in 5 ml of spinal fluid or saline solution through a lumbar puncture at the level of the fourth and fifth lumbar vertebrae. Usually three doses suffice but more may be required depending on the spinal fluid response. During intrathecal therapy oral amethopterin is discontinued.

**Results of Treatment—Prognosis for Survival** The beneficial effects of treatment may be measured by a comparison of the duration of life in the prehormonal era and in the posthormonal and chemotherapeutic era. In an analysis of cases<sup>2,11</sup> for the period in which no treatment was available, 50 per cent of the children with acute leukemia given supportive therapy not including antibiotics expired within a period of approximately four months after the onset of the first definite symptoms. The middle two thirds of patients survived from approximately two months to eight months and 10 per cent for as long as eleven months. In a smaller series,<sup>3</sup> 80 per cent treated with transfusions alone survived less than six months. In another untreated group comprised of children and adults with

gram of body weight in conjunction with parenteral ACTH and hydrocortisone therapy

**Hospitalization** Except for the most seriously ill patients for whom hospitalization is urgent the average patient with leukemia can be managed on an ambulatory basis. In patients with the milder disease for whom ACTH and the steroid hormones are chosen for initial treatment hospitalization is necessary because a precipitous drop in the white blood cell count frequently occurs soon after treatment is instituted necessitating adjustment of dosage.

Whether the leukemic child with even the mildest form of the disease should be treated from the first on an ambulatory program is debatable. In our experience an introductory period of hospitalization for about a week or ten days at the time of the diagnosis has proved extremely useful regardless of the chemotherapeutic agent that is chosen and the degree of severity. It permits a more adequate compilation of data including roentgenographic studies on the basis of which a program of treatment is planned.

When the antimetabolites are introduced initially severe myelotoxic effects may be manifested within the first two weeks of treatment in sensitive patients. Hospitalization during this period offers an opportunity for institution of adjuvant treatment especially in patients with prolonged bleeding.

More important is the opportunity which an initial hospital stay provides in helping parents adjust to the traumatic impact of a fatal childhood disease. Frequent interviews with the attending physician help to acquaint parents with miscellaneous details concerning the nature of the disease which they are anxious to ascertain. Following this hospitalization however ambulatory treatment is desirable unless serious clinical and hematologic relapses develop.

**Simplified Program of Treatment in Stem Cell (Lymphoblastic) Leukemia** The plan most often followed in our clinic for patients with stem cell leukemia is to initiate treatment with prednisone until a clinical and hematologic remission is established. During this period the bone marrow is examined weekly. With a remission at approximately three to four weeks the child is discharged without further antileukemic therapy. Strict attention is given subsequently to incipient signs of a relapse. These include pallor, fatigability, loss of appetite, bone pains, chilly sensations, easy bruising and beginning enlargement of the spleen. At this time the bone marrow is re-examined for confirmation of leukemic spread.

With the relapse methotrexate or 6-mercaptopurine is administered. With a subsequent relapse prednisone is reintroduced and within a few days either methotrexate or 6-mercaptopurine is substituted for the drug in previous use. In the absence of signs of toxicity from the antimetabolite prednisone is gradually withdrawn.

**Bone Marrow Transplantation** <sup>21, 22</sup> The demonstration that several species of animals given lethal doses of irradiation will recover after the infusion of normal bone marrow prompted trial of this procedure in human beings. Leukemia particularly has been the object of energetic clinical experimentation.

It is of interest that marrow injected intravenously seeks out the denuded marrow spaces and repopulates them. Theoretically total body irradiation destroys the immunologic defenses so that heterotransplants of normal human bone

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acute leukemia the figure of 50 per cent survival at four months is given<sup>11</sup>

In contrast to these groups is the effect on longevity of treatment with the antimetabolites ACTH and the adrenocortical hormones. With this program of treatment 25 per cent still succumb within the first six months although a small number survive beyond two years.<sup>1</sup> The increased survival in patients with the lymphoblastic type of leukemia which is prevalent in childhood has been emphasized especially in those patients with counts below 10 000 per cubic millimeter.<sup>12</sup> The results of treatment have been summarized as follows:<sup>1</sup> for patients treated before folic acid antagonists were used the 50 per cent survival time was four to five months as compared with 9.2 months when folic acid antagonists and/or adrenocortical steroids were used and 12.5 months since 6 mercaptopurine has been added.<sup>1</sup> Equally impressive is the striking clinical improvement in most patients which accompanies the hematologic remission induced by the newer therapy.

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liver lymph nodes and bone marrow rather than to have aggregated as nodular masses as they do in multiple myeloma.<sup>4-46</sup> Hyperglobulinemia and occasionally Bence Jones proteinuria can sometimes be present.<sup>46</sup> These cases are often difficult to differentiate from multiple myeloma which may also be accompanied by substantial numbers of plasma cells in the peripheral blood.<sup>6</sup>

**Bone Marrow Replacement and Leukoerythroblastosis** Space occupying lesions of the bone marrow resulting from infiltration with foreign cells or an overgrowth of fibrous or bony tissue are associated with a heterogeneous group of diseases. This process results in a chronic progressive anemia, a low or occasionally greatly elevated leukocyte count and the presence of varying numbers of immature myeloid and erythroid cells in the peripheral blood.<sup>47</sup> These conditions are accompanied by marked enlargement of the spleen and liver in which blood cells are produced which normally originate in the bone marrow. The resulting blood disorders have been described under the title of myelophthisic or leukoerythroblastic anemia and since anemia need not necessarily be present the blood response may be termed leukoerythroblastosis.

Leukoerythroblastosis is characterized by the presence of immature leukocytes of the myeloid series in the peripheral blood and nucleated red cells out of proportion to the degree of anemia. It may be provoked by invasion of the bone marrow by malignant cells of organs likely to metastasize to the bones, usually carcinomas. Children with lymphosarcoma and Hodgkin's disease may develop this type of blood response. The abnormal blood picture results from irritation or stimulation of normal adjacent marrow into excessive myeloid and erythropoietic activity.

**Myelofibrosis** The syndrome of myelofibrosis is a myeloproliferative process involving the bone marrow accompanied by extramedullary hemopoiesis and splenic enlargement. It occurs less frequently in children than in adults. The process may be either focal or generalized or primary or secondary and is of varying grades of severity. Myelosclerosis is characterized by increased production of fibrous (myelofibrosis) and bony tissue (osteosclerosis). Fibrosis and osteosclerosis of the marrow occur as a primary and idiopathic disease and in connection with such conditions as polycythemia in the course of the disease and in the "spent" phase leukemia, Hodgkin's disease, Gaucher's disease, xanthomatosis, tuberculosis, infections caused by chemical or physical agents and renal rickets.<sup>3</sup> A diverse terminology has been applied to the syndrome such as myeloid metaplasia of the spleen, leukoerythroblastic anemia, osteosclerosis, agnogenic myeloid metaplasia of the spleen and erythroleukemia (Di Guglielmo's syndrome).

Myelofibrosis is associated with slight to marked leukocytosis, a leukemoid reaction with early granulocytes, myelocytes and occasionally myeloblasts, variation in red cell morphology with normoblasts and teardrop shaped erythrocytes,<sup>6,34</sup> anisocytosis, poikilocytosis, variable platelet levels, circulating megakaryocytic fragments and tissue megakaryocytosis. The hemoglobin level is usually low but may be normal. Aspiration of a hypocellular bone marrow and difficulty in obtaining marrow at aspiration because of hardness of the bone indicate the presence of fibrous or other pathologic tissue. Dry taps are fre-

## *Leukemia—Allied Disorders*

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There are a number of conditions of a leukemic nature that occur infrequently which resemble the more common forms clinically and are characterized by an abnormal cell of an unusual type. Also allied to this unusual group of leukemias are the solid leukocytic neoplasms such as chloroma and the lymphomas in which the process is aleukemic in a few sites at the onset but which as it becomes generalized may simulate the typical blood and bone marrow picture of leukemia.

*Infrequent Types of Leukemia* Among the unusual types of leukemia and extremely uncommon in children are the mast cell basophilic and plasma cell leukemias.

*Mast Cell Leukemia* Mast cells which have been shown to produce the characteristic skin lesions in urticaria pigmentosa can occasionally infiltrate many viscera and supporting tissues. Ellis<sup>1</sup> described the involvement of the skin, bone marrow, mesenteric lymph nodes, spleen, and liver in a 1 year old infant. Bone marrow involvement is reflected in the roentgenogram by marked thickening and increased density especially of the frontal bone of the skull and coarsening of the trabeculations and thinning of the cortices in the small and large tubular bones.<sup>48</sup> Conditions in which there is splenic involvement with a generalized visceral distribution of cells, hepatosplenomegaly, the appearance of mast cells in the peripheral blood, anemia, and at times thrombocytopenia have been classified as mast cell leukemia.<sup>9, 49, 50</sup> The difficulty of separating the mast cell from the blood basophil is reflected in the patient in whom tissue and marrow mastocytosis is associated with blood basophilia. The differentiation between the tissue mast cell and basophilic leukocyte is described elsewhere (Chapter 18).

*Basophilic Leukemia* Increased numbers of basophilic cells have been described in the course of chronic myelocytic leukemia. In one instance of acute leukemia in a 5 month old infant, mature basophilic leukocytes and myelocytes predominated.<sup>38</sup> In another case in a young adult, mature basophilic cells characterized the blood smear but were not associated with immature forms.<sup>8</sup>

*Plasma Cell Leukemia* Cases of leukemia have been described in which plasma cells appeared in large numbers in the peripheral blood accompanied by anemia and leukocytosis. These cells were found to have diffusely infiltrated the spleen

megakaryocytosis in the hemitopoietic tissues or with myeloclerosis.<sup>4</sup> Extramedullary megakaryocytosis is less efficient in the formation of platelets than is the bone marrow and accounts for the discrepancy between the platelet count and the number of megakaryocytes in the metaplastic organs.<sup>4</sup> Whether the condition can be categorized as megakaryocytic leukemia in the absence of invasion of these cells into the blood stream is still controversial.<sup>10</sup>

**Thrombocythemia** Megakaryocytic hyperplasia of the bone marrow with markedly increased platelet counts (thrombocythemia) is regarded as a proliferative disorder of the bone marrow. Primary<sup>46</sup> and secondary types have been recognized. Secondary thrombocythemia occurs in association with an underlying condition such as carcinoma, myelogenous leukemia, polycythemia, and Hodgkin's disease and following splenectomy. It has also been described as an idiopathic disease with prolonged bleeding time, venous thromboses, splenomegaly, spontaneous hemorrhage, and a normal coagulation time. The cause of the hemorrhage and prolonged bleeding time is not known.<sup>4</sup>

**Erythremic Myelosis (Di Guglielmo's Disease)** Erythremic myelosis is a rare disease described by Di Guglielmo<sup>48</sup> which is characterized by erythropoietic hyperplasia within the bone marrow and extramedullary sites. Irregular remittent fever, splenomegaly proportionally greater than hepatomegaly, thrombocytopenia, granulopenia, and anemia are associated with enormous numbers of erythroblasts in the peripheral blood which are basophilic, multinucleated, or otherwise atypical.<sup>49</sup> Both the bone marrow and the peripheral blood show overcrowding with erythroid elements which become progressively immature. This neoplastic disorder affecting erythropoietic tissue is analogous in its cellular specificity to involvement of myeloid cells in leukemia. Cases have been reported in patients in younger age groups,<sup>41-61</sup> as well as in adults. In contrast to hemolytic anemia which it resembles, the reticulocyte count is low and remains so. The disease occurs as a short and acute form of several months duration or a chronic and prolonged form lasting up to two years. Transfusions, splenectomy, and steroids have been employed in treatment, but the disease is ultimately fatal with hemorrhagic manifestations.

A strongly positive periodic acid-Schiff (PAS) reaction (for glycogen and mucopolysaccharides) occurs in many of the erythroblasts from the bone marrow and blood of patients with Di Guglielmo's disease.<sup>47</sup> A strongly positive reaction also occurs in many erythroblasts of patients with iron deficiency anemia and thalassemia major. Erythroblasts are uniformly negative in the bone marrow of normal persons and in that of patients with pernicious anemia, nutritional macrocytic anemia, aplastic anemia, and polycythemia.

Erythroleukemia is a condition in which hyperplasia of erythroblastic and leukoblastic tissues are combined. The peripheral blood and bone marrow reveal an admixture of myeloblasts and immature erythroblasts.<sup>8</sup> According to another concept, erythremic myelosis may eventually emerge as leukemia, particularly when life is prolonged by intensive transfusion therapy.<sup>11</sup> In one such patient the anemia was characterized by intensive erythroblastemia, marrow erythroblastosis, and a hemorrhagic state. At death the patient

quently encountered in attempts to aspirate the marrow.<sup>2</sup> Bone marrow biopsy which is frequently necessary reveals patchy myelofibrosis hyperplastic foci of myelopoiesis and often increased numbers of megakaryocytes. Roentgenographic examination reveals changes suggestive of osteosclerosis in variable parts of the skeleton such as the ribs pelvis spine and femora. As the disease progresses the liver and spleen enlarge and anemia develops. Evidence of increased hemolysis is indicated by fecal urobilinogen reticulocyte and red cell survival studies.<sup>3</sup>

Although fibrosis of the bone marrow with extramedullary hematopoiesis usually occurs in adults such cases have been reported in pediatric patients. The findings in the latter also consist of fibrosis of the bone marrow hepatosplenomegaly with immature granulocytic and erythrocytic elements in the peripheral blood and a predominance of megakaryocytes in the spleen and bone marrow.<sup>4,5</sup>

Treatment consists of transfusions and splenectomy when blood requirements are excessive. Despite the apparent need for the spleen as a source of extramedullary hematopoiesis splenectomy has often been effective when the hemolytic component accompanies its enlargement. Preliminary treatment with ACTH or cortisone has been employed to determine whether it will stimulate red cell production decrease the rate of hemolysis or both. Splenectomy is not undertaken until full evaluation is made especially for the presence of islands of red cells and megakaryocytes in the bone marrow and possibly in the liver. The prognosis is interrelated with the underlying disease.

**Osteopetrosis (Marble Bone Disease, Albers-Schönberg Disease)** Osteopetrosis is a rare disease characterized by increased thickening and density of the cortical and spongy portions of the entire osseous system.<sup>3,6</sup> The onset is usually in fetal life less often in later childhood. The bones are rigid and brittle and there is a tendency for spontaneous fractures and slipping of the epiphyses to occur. The individual bones including those of the skull appear opaque heavy and lacking in finer structure and in the roentgenogram normal markings are obliterated. Hydrocephalus is not uncommon in infancy. The disease follows a strong familial and hereditary pattern.<sup>4,6</sup> It has been attributed to a failure of resorption of the calcified cartilaginous matrix during endochondral bone formation or to the influence of an unknown agent which damages the bone-forming blastema.<sup>6,7</sup>

The clinical picture includes anemia splenomegaly enlargement of the liver and body deformities. Anemia is common but not invariable and is associated with a leukoerythroblastic response. Normoblasts and myeloid cells appear in the peripheral blood in some patients resembling myeloid leukemia. Thrombocytopenia may be marked and associated with massive hemorrhage. No treatment is of benefit in this disease. Transfusions are given as required.

**Extramedullary Megakaryocytosis and Acute Megakaryocytic Leukemia** Hypertrophy of the bone marrow with respect to myeloid erythroid and megakaryocytic cells has been described in which the megakaryocytes predominate.<sup>1,8</sup> The term megakaryocytosis in contrast to the term leukocytosis applies to an increased number of megakaryocytes in any tissue intramedullary or extramedullary usually the latter. Megakaryocytes appear with an aleukemic or subleukemic blood picture often with chronic myelogenous leukemia with



Symptoms include loss of weight and appetite weakness fever cough, and dyspnea In a majority of patients the disease progresses through a latent period which is often protracted and during which nodes enlarge without symptoms This period is followed by one of further progressions and generalization during which symptoms of varying severity appear with cachexia as a terminal symptom<sup>31</sup> Fatigue anorexia, loss of weight, and intermittent fever (Pels-Ebstein) herald or accompany the disease The spleen is enlarged in about 60 to 70 per cent of patients with advanced disease<sup>3</sup>

The causative agent is unknown although viral and bacterial, especially tuberculous etiologies have been suggested but not fully established. Inflammatory and neoplastic theories have also been advanced but unproved The disease affects males more often than females with a predominance in the ratio of 3 to 1. It is most common in adults between 20 and 40 years of age and comparatively rare in children, in whom it occurs between 4 and 12 years of age and even earlier<sup>32</sup>

The nodes involved in order of prevalence are the cervical, axillary inguinal mediastinal, and mesenteric nodes The cervical nodes are involved in 50 to 75 per cent of all patients The nodes are usually discrete and the capsule is rarely infiltrated. The essential histologic features of Hodgkin's disease are the Reed Sternberg cells pleomorphism of the cellular tissue the presence of eosinophils and fibrosis<sup>3</sup> The pathologic changes vary from patient to patient and in different areas in the same patient The diagnosis is based on the distinctive histopathologic alteration in an involved area The microscopic picture is characterized by a proliferation of the large reticuloendothelial cells replacing the lymphoid tissue and the presence of giant cells derived from the reticulum cells The latter are the nucleated and multinucleated Reed Sternberg cells the pathognomonic cell and essential diagnostic feature of Hodgkin's disease The large nucleus of the Reed Sternberg cell has a well-defined membrane with many folds and outcroppings<sup>33</sup> Nucleoli are especially prominent The diagnostic lesions consist of eosinophils lymphocytes endothelial cells Reed Sternberg cells myelocytes and megakaryocytes Eosinophils may be especially prominent.

In the later stages the usual structure of the lymph nodes disappears and is gradually replaced by fibrous tissue From the initial manifestation of Hodgkin's disease as a painless swelling of one or more groups of superficial nodes the disease progresses Mechanical pressure of the enlarging nodes produces local clinical manifestations such as dyspnea from tracheal and mediastinal involvement. The disease may become actively invasive however extending beyond the confines of the lymph nodes to involve the spleen liver bones lungs and, to a lesser extent, other organs Radiation results in increased fibrosis and matting together of the nodes The disease is uniformly fatal within a period of a few months to several years.

*Lymphosarcoma* The term lymphosarcoma has been used to include all primary malignant tumors of lymphoid origin except Hodgkin's disease and has been subdivided into giant follicular lymphosarcoma, reticulum cell sarcoma, and lymphosarcoma.<sup>34</sup> In its restricted terminology the predominant cell in lymphosarcoma corresponds to the small mature lymphocyte so that the condition

was found to have changes characteristic of acute granulocytic leukemia<sup>11</sup>

**Chloroma and Chloroleukemia** In rare cases of acute leukemia in children and young adults there are coexisting localized tumor masses found in close relationship to the periosteum of the bones of the face ribs sternum or vertebrae and less commonly in the viscera. These tumors are referred to as chloroma because of a pale greenish color on the cut surface which fades rapidly on exposure to light and air. The nature of the pigment causing the green color is still controversial with theories involving white blood cell and hemoglobin derivatives. These tumors consist of primitive white blood cells usually myeloblasts less commonly myelocytes and monocytes. Clinically the classical case of chloroma is characterized by an orbital tumor frequently causing exophthalmos and proptosis lymphadenopathy and a rapidly progressing anemia associated with acute myelogenous and monocytic leukemia.<sup>12</sup> A congenital case has been reported in a 13 day old infant.<sup>13</sup>

**Neoplasms of Lymphoid Tissue (Malignant Lymphomas)** The malignant lymphomas include Hodgkin's disease and lymphosarcoma. In this classification are included diverse conditions of unknown etiology arising from lymphoid tissues. The cellular constituents found in the lymph nodes and lymph follicles form the basis of specific conditions—namely lymphoblast lymphocyte reticulum cell and plasma cell. Single cells alone may be involved or the histologic patterns may be more complex with the presence of nodules or granulomatous lesions of several cell types. Exact diagnosis depends upon the identification of distinctive cells or a group of cells. It is often impossible to distinguish between these disorders without access to complete clinical data multiple laboratory aids such as roentgenograms bone and lymph node biopsy bone marrow aspiration and blood studies. Even with these facilities diagnosis is often difficult and depends upon clinical and laboratory changes during the course of the disease. Transitions are known to occur from one histologic type of neoplasm to another and several different microscopic patterns are often present in the same patient and same lymph nodes.<sup>10</sup>

**Hodgkin's Disease** Hodgkin's disease is a fatal disease characterized by a painless and progressive enlargement of the superficial and deep lymph nodes and often of other lymphoid structures of the body and of the spleen. It is protean in its manifestations and during its course can resemble both tumor and infections.<sup>14</sup> As in patients with lymphosarcoma the first evidence of Hodgkin's disease may be detected in the form of a mediastinal mass in the chest observed by roentgenographic examination.<sup>15</sup> However in contrast to lymphosarcoma in which symmetrical areas are attacked in the early stages of Hodgkin's disease the pathology is usually unilateral.

Hodgkin's disease is classified as paraneoplasia granuloma or sarcoma. The paraneoplasia involves chiefly lymph nodes in which the normal architecture may or may not be altered. The granuloma type represents the classic form of the disease in which there is a gradual loss of normal architecture of the lymph nodes and replacement by pleomorphic cellular tissue. Hodgkin's sarcoma represents the most invasive and destructive form of the disease in which cells larger than lymphocytes predominate normal architecture of the nodes is destroyed and necrosis is present.<sup>13</sup>

lymphosarcoma cell<sup>30</sup> is the nucleolus which stands out as a sky blue round area surrounded by a deep blue black run of chromatin. In the course of lymphosarcoma the bone marrow may become heavily infiltrated with lymphosarcoma cells without their appearance in the blood stream. It may be that death ensues before the peripheral blood is invaded.

We have seen a patient in whom this process appeared to have been reversed. In an 11 year old child with anemia, thrombocytopenia and splenomegaly the bone marrow and to a lesser extent the peripheral blood were heavily infiltrated with blast cells. On the basis of a diagnosis of lymphoblastic leukemia steroids were administered and a complete remission was obtained which persisted for fifteen months. At this time the onset of abdominal pain led to the discovery of a retroperitoneal lymphosarcoma. Radiotherapy produced a prompt resolution of the mass. One month after the beginning of this treatment leukemic cells were again found in the blood and bone marrow. The initial leukemic cells may have represented metastasis from a quiescent focus of lymphosarcoma which later became reactivated.

Massive enlargements of the mediastinum causing circulatory or respiratory distress which precede the blood picture of acute leukemia have been reported in children. Whether the mass is of thymic origin exclusively or associated with mediastinal lymph nodes is not certain. In these patients irradiation with the roentgen ray produces marked relief from the shortness of breath. The mediastinal tumor disappears completely and permanently within a few days although patients have been known to succumb later to acute leukemia.<sup>7</sup> Roentgen therapy as a factor in precipitating the leukemic phase may have been overemphasized however.<sup>4</sup>

**FOLLICULAR LYMPHOSARCOMA (GIANT FOLLICULAR LYMPHOBLASTOMA, BRILL SYMMERS DISEASE)** This type of lymphosarcoma involves the lymph nodes, spleen and other lymphoid tissues and is characterized by a prominence of the follicular structure. Clinically it consists of generalized lymphadenopathy, splenomegaly, delayed appearance of anemia, tendency toward pleural and peritoneal effusions, bone involvement and unilateral exophthalmos. Hyperplasia of lymph follicles and malpighian bodies with an increase in these elements numerically and in size is a striking feature. As the disease progresses follicular hypertrophy and hyperplasia are replaced by lymphosarcoma, lymphocytic leukemia or rarely Hodgkins disease. The disease is of long duration and is remarkably sensitive to a ray therapy more so than any other form of lymphoma. This is particularly true in the earlier phase but less so in the later malignant phases.

**Blood Changes in the Lymphoma Group** The degree of anemia and the white blood cell and platelet levels show great variation in patients with the malignant lymphomas. In patients with the early stages of lymphosarcoma and in those with follicular lymphoblastoma the anemia may be absent or slight but eventually progresses. In patients with Hodgkins disease the anemia usually is normochromic and normocytic. Various mechanisms may be involved in producing the anemia. Prior to the advent of the Coombs test and techniques to determine red cell survival the anemia in patients with this group of diseases and in those with leukemia was ascribed to crowding out of the marrow by pathologic cells.

is often termed small cell lymphosarcoma. In the reticulum cell sarcoma the typical cell is the reticulum cell which is more than one and a half times the size of the mature lymphocyte. Reticulum cell sarcoma mimics lymphosarcoma and is regarded as its most undifferentiated type. Lymphosarcoma and reticulum cell sarcoma can be discussed together from the clinical, pathologic and therapeutic standpoints although the latter is generally more highly malignant and invasive and metastasizes even more readily than the lymphosarcoma.<sup>1</sup>

Lymphosarcoma occurs with greater frequency in adults than in children but in sufficient numbers in the latter to prompt a review of its clinical and hematologic aspects. In a series of 583 children with malignant tumors treated at the Memorial Center twenty seven (5 per cent) had lymphosarcoma.<sup>1</sup> The malignant neoplasms comprising this group are often difficult to classify, but orientation is facilitated by considering the clinical features together with an interpretation of the specific cellular components. In addition to lymphocytic and reticulum cell forms arising from the periphery and germinal center of the lymph follicle respectively, lymphoblastic and mixed lymphocytic and reticulum cell types have been described.

These tumors of the lymphosarcoma group originate within and outside the lymph nodes in the many anatomic regions in which lymphoid tissue is present. Expansion of these areas by overgrowth of malignant cells results in destruction of the normal architecture of lymphoid tissue and infiltration of surrounding tissues. The disease therefore may be limited initially to a single locus or to a region or may be generalized with constitutional symptoms and signs.<sup>14</sup>

Symptoms and signs originate in the area of primary involvement or from the areas invaded by direct extension from affected lymph nodes. The eyes, nervous system, skin, thymus, gastrointestinal tract and cervical, mediastinal, mesenteric and retroperitoneal nodes serve as primary sites. The anterior mediastinum is frequently involved.

In an appreciable number of patients lymphoblastic leukemia develops during the course of lymphosarcoma, either following roentgen irradiation or occurring spontaneously, regardless of the site involved. Of a group of forty two children with small cell lymphosarcoma 9 to 21.4 per cent showed a transition into leukemia.<sup>15</sup> To this association with a leukemia component the term leukosarcoma has been applied. In a series of 113 patients with documented cases of reticulum cell sarcoma the course in six terminated in a syndrome resembling acute leukemia with a high percentage of immature cells in the blood and bone marrow.<sup>16</sup>

The actual time relationship to the development of leukemia is not always apparent for examination of the blood smear may reveal a small percentage of abnormal cells at any stage of the disease. These cells however may represent tumor cells rather than actual lymphoblasts. Attempts have been made to differentiate between the typical lymphoblast of stem cell leukemia and the lymphosarcoma cell reaching the blood stream by invasion from the primary focus. One of the main features of the lymphosarcoma cell is the coarsely reticular and more deeply staining chromatin as compared with the leptochromatic or more lightly staining nucleus of the lymphoblast. A distinctive feature of the

lymphocytic tumor cell infiltrates may be found. In patients with Hodgkin's disease myeloid and megakaryocytic hyperplasia is common. In patients with this disease reticulum cells, plasma cells, polymorphonuclear leukocytes, and lymphocytes are increased, and in many eosinophils are conspicuous. In rare cases cells resembling Reed-Sternberg cells are found in bone marrow aspiration.<sup>3, 9</sup> These must be distinguished from the neighboring megakaryocytes which they resemble. The cells appear as hypertrophied reticulum cells with one or several very large blue nucleoli; in other cells a large number of nuclei are grouped together forming a giant cell. The presence of giant nucleoli which stain pale to deep blue with Romanowsky stains serves as a distinguishing feature from megakaryocytes in which the nucleoli are small and inconspicuous and present only in the youngest cells.<sup>10</sup> In a recent case in a boy 15 years of age these abnormal cells as well as a marked increase of eosinophils were found in the bone marrow. The chief complaint was abdominal pain and marked loss of weight. The lymph nodes were not enlarged and the spleen was only slightly increased in size.

Lymphosarcoma and reticulum cell sarcoma can be identified more frequently in bone marrow specimens.

**Treatment.** The malignant lymphomas may be considered as a group with regard to the general principles of treatment. Lymphosarcoma and Hodgkin's disease require special consideration since they figure more prominently in pediatric practice. Surgery, roentgen ray irradiation, and chemotherapy together with supportive treatment (including transfusions and antibiotics) constitute the agents of direct attack upon these diseases.

The choice and intensity of therapy depends upon whether the disease is strictly localized or is widespread. Surgical excision has been recommended in the few patients in whom the lesion is confined to a single accessible focus and in whom complete examination, including roentgenograms of the chest, skeleton, and gastrointestinal tract, reveals no other involvement.<sup>9</sup> Excisions are followed postoperatively by roentgen ray irradiation.

Roentgen ray therapy, however, represents the most effective and dependable suppressive agent, especially for localized lesions.<sup>14</sup> It is often used alone initially or in conjunction with chemotherapy, especially when patients are no longer responsive to roentgen irradiation alone. Among the chemotherapeutic agents the polyfunctional alkylating agents, nitrogen mustard (HN) and its derivatives constitute essential means of treatment, especially in patients with Hodgkin's disease. The use of nitrogen mustard with roentgen ray irradiation of selected areas constitutes an effective treatment, especially in patients with disseminated lesions. The sequence with which they are administered varies with each patient and frequently with special experience of a clinic.

In lymphosarcoma in childhood roentgen ray therapy constitutes the mainstay of treatment. Also useful are the antimetabolic drugs, chiefly folic acid antagonists such as amethopterin (Methotrexate). (The dosage of amethopterin and other antimetabolic drugs will be found in the discussion on the treatment of leukemia, Chapter 23.) Nitrogen mustard and related compounds have little if any lasting benefit.<sup>20</sup>

On the basis of immunologic studies the mechanism of hemolysis in many of these patients has now been shown not to differ significantly from that of patients with idiopathic autoimmune hemolytic anemia.<sup>51</sup> Anemia, reticulocytosis, spherocytosis, bilirubinemia of the direct type, and a positive Coombs test are conspicuous. In a small number of patients autoimmune antibodies are not detected and the signs of hemolysis are slight or absent. In the latter the anemia is normochromic, there are no abnormalities in the red cell morphology, and transfusion requirements are occasionally increased.<sup>52</sup> In patients with either type red cell survival is decreased. Steroids act favorably in patients with autoimmune hemolytic anemia, but there is no response in those with the contrasting type.

In another study no shortening of the life span was apparent until the hemoglobin level dropped below 7.5 gm per 100 ml, and inadequate erythropoiesis was a major cause of anemia in patients with a negative Coombs test.

The leukocyte count in patients with Hodgkin's disease may be normal, low, or moderately increased. Leukopenia is uncommon, however, and lymphocytosis is rare.<sup>53</sup> The white blood cell count in this disease usually ranges from 10,000 to 15,000 per cubic millimeter but may reach levels of 30,000 per cubic millimeter or more. The higher counts usually accompany the late stages of the disease. The differential count reveals a tendency toward polymorphonuclear predominance, ranging from 85 to 90 per cent. Monocytosis, eosinophilia, and lymphocytopenia characterize the peripheral blood of the patient with Hodgkin's disease. Extreme degrees of eosinophilia are infrequent.

The white blood cell count in patients with lymphosarcoma and follicular lymphoblastoma may be normal, or there may be relative or absolute lymphocytosis. Tumor cells of lymphosarcoma may be found in the blood smear. Both lymphosarcoma and follicular lymphoma may be associated with lymphocytic leukemia during the progression of each disease. Hematogones have been found in increased numbers in the peripheral blood of patients with follicular lymphoblastoma. These are cells smaller than lymphocytes with nuclei composed of dense chromatin and almost devoid of cytoplasm, so that the cells give the appearance of naked nuclei. An indentation or a horizontal crack through the cell may be seen. Leukemoid blood pictures have been observed occasionally in all members of the lymphoma group.

The platelet count in patients with Hodgkin's disease is usually normal. Markedly increased numbers of platelets and bizarre forms occasionally present reflect the excessive numbers of megakaryocytes in the bone marrow. The platelet count in patients with lymphomas other than Hodgkin's disease is usually normal, or occasionally reduced. Thrombocytopenia in patients with lymphomas, including those with Hodgkin's disease, may represent the effects of a pathologically involved spleen exerting a hypersplenic effect.

Abnormal and primitive reticulum cells with morphologic features of Reed-Sternberg cells have been described in the peripheral blood of a patient with Hodgkin's disease.<sup>54</sup>

**Bone Marrow Changes.** It has been stated<sup>55</sup> that bone marrow aspiration is of greatest diagnostic value in patients with lymphosarcoma in whom abnormal

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Nitrogen mustard and its derivatives possess the ability to arrest mitosis and owe their effectiveness to their toxicity for all cells but more so to bone marrow lymphoid and reticulo endothelial tissues. With these compounds improvement sets in within a few days with subsidence of fever and shrinkage of lymph nodes and spleen and persists from periods of a month to a year or longer. Nausea vomiting and depression of the bone marrow with pancytopenia are likely to result from use of these compounds. Leukopenia thrombocytopenia and anemia may become marked within a few days to a week following treatment and persist from three to five weeks. Rigid attention therefore should be given to blood studies before and after treatment especially with regard to leukopenia and thrombocytopenia. This caution is particularly applicable when successive courses of nitrogen mustard ( $\text{HN}_2$ ) are contemplated. In general it is inadvisable to employ this form of treatment when the white blood cell count is below 3 500 per cubic millimeter.

HN (nitrogen mustard) the principal member of this group of drugs is given intravenously in a total dosage of 0.4 mg per kilogram of body weight. It may be divided in a fractional dosage of 0.1 mg per kilogram in four consecutive days when there is evidence of abnormal depression of the bone marrow and widespread disease. The calculated dose is injected into an intravenous infusion of physiologic saline solution or 5 per cent glucose in water.

TEM (triethylene melamine) a compound with nitrogen mustard activity has the advantage of oral administration permitting ambulatory treatment. The dosage is 0.12 mg per kilogram orally. A single course consists of 10 mg divided into 2.5 mg doses daily for four days.<sup>14</sup> The restoration to normal white blood cell and platelet counts is a prerequisite for repetition of treatment.

Chlorambucil (Leukeran CB 1348) one of the newer nitrogen mustard derivatives is available in 2 and 4 mg tablets. It is given orally in a dosage of 0.2 mg per kilogram of body weight. With proper adjustment of dosage chlorambucil has been found as effective as other alkylating agents in the treatment of patients with Hodgkin's disease lymphosarcoma chronic lymphocytic leukemia and also chronic myelocytic leukemia.<sup>44</sup> To achieve maximum results the drug is given to the point of early bone marrow depression. Close observation of the hemoglobin level white blood cell count and platelet level is mandatory. Chlorambucil is given initially in a dosage of 0.1 mg per kilogram when the bone marrow function has been previously impaired by radiation or chemotherapy.

ACTH and the adrenocortical steroids known to cause lympholysis are useful when the disease is generalized and constitutional symptoms are present. They also are important accessory therapeutic agents with the development of an acquired hemolytic anemia. The antifols and 6 mercaptopurine have also been found useful especially in patients with lymphosarcoma when the disease has become generalized.<sup>1</sup>

Hemolytic anemia may be so severe and splenomegaly so marked in patients with lymphosarcoma that splenectomy may be required in spite of continued transfusion and steroid administration to achieve stability of the hemoglobin level.

With the combination of therapeutic agents the duration of life of patients with the lymphomas can be extended to many years. Survivals of three and five years and longer have been reported during which the patient appeared in good health.<sup>1</sup> In children however the course is shorter and the disease more aggressive than in adults. Despite periods of remission recurrences are inevitable and inevitably a fatal outcome ensues.

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## Disorders of the Spleen and the Reticuloendothelial System

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**Role of the Spleen in Blood Disorders** The spleen plays an essential part in the pathogenesis of so many blood disorders that a review of its structure and function serves as a frame of reference for appraising deviations from the normal.

**Structure of the Spleen** The spleen is structurally organized to perform its main physiologic functions of blood formation, sequestration and destruction of blood cells, principally the erythrocytic series, and protection against infection. It possesses a smooth muscle capsule and trabeculae permitting its contraction. A vascular system with connecting sinusoids allowing withdrawal of cells from the circulation, a lymphoid system (malpighian corpuscles) corresponding to similar tissue elsewhere in the body, and a rich supply of reticuloendothelial tissue. Elements of the reticuloendothelial system are either scattered through the pulp or line the vascular and lymphatic sinusoids. Whether free or fixed, these cells exert a phagocytic action. Mesenchymal cells and lymphoid tissue serve as a source of monocytes and lymphocytes. The sequestration of blood into pulp spaces and venous sinuses exposes bacteria and particulate matter to phagocytosis by the reticuloendothelial tissue and at the same time promotes the stasis and destruction of red blood cells.

**Normal Functions of the Spleen** The normal functions of the spleen include the production, storage and destruction of blood and protection against infection.

**Blood Production** The spleen is one of the principal sites of blood formation from the second to the fifth month of fetal life. After passing through the hemocytoblast stage, mesenchymal cells in the fifth month give rise to erythroid blasts. After the fifth month, red cell production diminishes and is absent by the sixth month.<sup>1</sup> Lymphocytes are produced in the spleen mainly in the white pulp (lymphatic tissue, malpighian bodies). According to the unitarian theory, monocytes are derived from lymphocytes upon migrating into the red pulp. In stress situations such as hemorrhage, hemolysis and leukoblastic infiltration in infants and children, fetal blood foci are reactivated with the resumption of hematopoiesis. This function by which the spleen, liver and lymph nodes revert to their fetal function of hematopoiesis is known as extramedullary

hematopoiesis and applies equally to the production of red blood cells, granulocytes and platelets. The relationship of the spleen to blood formation is further confirmed by the observation that in adult mice recovery of blood forming tissues after total irradiation is accelerated by previously shielding the surgically exteriorized spleen with lead.<sup>3</sup>

*Splenic control of normal maturation of the red cell surface* is indicated by the loss of stickiness of the reticulocytes during maturation, the loss of reticulum and shrinkage of diameter and volume due to loss of water.<sup>4</sup> This function is lost after splenectomy. Evidence of splenic control over the bone marrow is also suggested by the peripheral blood changes occurring after splenectomy—namely prompt increase in the number of white blood cells, platelets, nucleated red cells and target cells, tendency toward thinness of the red cells, decreased osmotic fragility and appearance of siderocytes, Heinz bodies and red cells containing nuclear fragments (Howell Jolly bodies).<sup>69</sup>

*Blood Storage* Although the spleen serves as a reservoir of red blood cells in the dog, cat and horse, evidence indicates that blood reservoirs of this nature do not exist in man.<sup>4</sup> In the average sized adult the spleen holds 20 to 30 ml of red blood cells.<sup>1</sup> During the passage through the spleen even normal red blood cells are rendered more fragile and a mild degree of spherocytosis is attained. It has been estimated that of the 120 day life cycle of the human red blood cell, about two days are spent in the spleen.<sup>17</sup> However, in patients with pathologic conditions, significant withdrawal of red blood cells from the circulation may take place, producing sudden severe anemia.<sup>47</sup>

*Blood Destruction* Through the reticuloendothelial system the spleen removes worn out and fragmented cells and red cells sensitized by agglutination, resulting in degradation of hemoglobin and formation of bile pigment. That abnormally shaped red cells are trapped and destroyed by the normal spleen is illustrated by the fate of the spherocytes in patients with hereditary spherocytosis.

*Protection Against Infection* It has been pointed out that the spleen, with its abundant content of macrophages, plasma cells and lymphocytes, is strategically located in the direct stream of the circulating blood for effective phagocytosis and antibody production.<sup>4</sup> The extent to which the spleen participates in antibody response is estimated by comparing the normal with the splenectomized animal and human being after the injection of microorganisms and other antigens. It has been shown that in the rabbit, abolition of antibody formation by roentgen rays may be prevented by shielding the surgically mobilized spleen.<sup>30</sup> A comparison of the antibody response to different types of agents in the splenectomized and nonsplenectomized animals and human beings has led to divergent results.<sup>4, 6, 64, 65, 71</sup> These differences may be due to the need for intravenous<sup>64, 65</sup> rather than subcutaneous<sup>66</sup> injections to demonstrate the depression of antibody response following splenectomy. There is satisfactory evidence that filtration of organisms or other particulate material takes place from the blood stream by the local phagocytic action of the macrophages in the spleen. Evidence for such a protective mechanism is suggested by experiments<sup>9</sup> in which intravenously injected bacteria cleared from the blood were recovered in the macrophages of the reticuloendothelial tissues of the liver and spleen.

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## 3 Miscellaneous

## A Rupture of spleen

## B Cysts

## C Tumors (i.e. lymphosarcoma and follicular lymphoblastoma)

## D Selected cases of hypoplastic anemia (including pure red cell anemia)

In a few cases characteristically severe and fulminating infections have followed splenectomy in children. Although the incidence is avowedly small in comparison with the ever increasing number of splenectomies close supervision of the splenectomized child is necessary for several years postoperatively until further information is obtained. Such patients should receive immediate and energetic treatment in the event of sudden and severe illness.<sup>71</sup>

## DISORDERS OF THE SPLEEN

*Splenomegaly*

Elsewhere (Chapter 15) it was stated that splenomegaly is associated more commonly with the hemolytic than with other anemias. In young children with anemia of comparable severity the spleen is more likely to be enlarged in those with severe Cooley's anemia than in those with sickle cell anemia or hereditary spherocytosis. The enlarged spleen in the patient with sickle cell anemia becomes progressively smaller and more fibrotic as the patient gets older. In interpreting the pathologic significance of an enlarged spleen it is important to remember that the spleen may be palpable in normal infants and children and at times does not recede in size until puberty. At birth the weight of the spleen is approximately 10 gm; at 1 year of age approximately 30 gm; at 6 years of age about 55 mg; at puberty approximately 95 gm; and in the adult about 155 gm.<sup>72</sup> It is estimated that the spleen must be enlarged two and a half to three times the normal size to be palpable. As is shown in the following list splenomegaly accompanies both hematologic and nonhematologic disorders.

## 1 Blood dyscrasias

## A Hemolytic anemias

- (1) Acquired hemolytic anemia
- (2) Hereditary spherocytosis
- (3) Severe Cooley's anemia
- (4) Sickle cell anemia
- (5) Many of the hemoglobinopathies

## B Leukemia

## II Infiltrative

## A Lipid

- (1) Crocker's disease
- (2) Niemann-Pick disease

## B No lipid

- (1) Letterer-Siwe disease (nonlipid reticuloendotheliosis)

## C Amyloidosis

## 3 Vascular

## A Chronic congestive splenomegaly (Banti's syndrome)

## B Chronic passive congestion

## 4 Infectious

Trapping of organisms by removal mechanisms of the liver and spleen have been demonstrated for staphylococci and *Escherichia coli* <sup>9 9</sup>

Properdin a natural serum protein which (in association with magnesium and complement) is involved in the destruction of selected bacteria and viruses has been found to be in lower concentration in splenectomized persons than in normal persons <sup>17\*</sup>

**Splenic Aspiration** <sup>14 83</sup> Splenic aspiration serves as an aid in hematologic examinations in patients with undiagnosed splenomegaly to determine the nature of the abnormal cell responsible for the condition. It has been helpful in the diagnosis of lymphoma, myeloid metaplasia kala azar, and Gaucher's disease especially when these conditions are suspected and bone marrow aspiration has been negative or inconclusive. Aspiration of the spleen also permits observations on its pathophysiology in patients with the hemolytic disorders and in those with hypersplenic syndromes. Cooperation of the patient is essential in carrying out this procedure because of the danger of excessive hemorrhage. It is therefore contraindicated in patients with a hemorrhagic tendency.

**Adrenalin Test in Diagnosis of Hypersplenic Syndromes** The adrenalin test has been employed to confirm the diagnosis of various hypersplenic syndromes with a depletion of one or more cells in the peripheral blood. Epinephrine is said to prompt the mobilization of sequestered cells into the peripheral blood. In one investigation <sup>13</sup> it was shown that this test provided only indirect corroborative evidence of the functional status of the blood-forming tissues. In splenomegalic conditions for instance there was no correlation between the degree of splenic contraction and the degree of hemapoietic response.

**Indications for Splenectomy** Splenectomy is most successful in patients with hereditary spherocytosis and to a lesser extent in those with thrombocytopenic purpura. By removing an inhibitory or destructive influence in patients with secondary hypersplenism it frequently restores the blood count to normal either in part or completely. As mentioned elsewhere (Chapter 16) in patients with severe Cooley's anemia the beneficial effects of splenectomy result in the elimination of a hemolytic factor and in a material decrease in transfusion requirements. With respect to the following conditions splenectomy is discussed in connection with management of the individual disorders.

- I Hemolytic disorders
  - A Hereditary spherocytosis
  - B Acquired hemolytic anemia
  - C In selected cases
    - (1) Thalassemia major (Cooley's anemia)
    - (2) Sickle cell anemia
- 2 Hypersplenic syndromes
  - A Without splenomegaly
    - (1) Chronic idiopathic thrombocytopenic purpura
  - B With splenomegaly
    - (1) Banti's syndrome
    - (2) Splenic neutropenia
    - (3) Splenic hematopenia
    - (4) Gaucher's disease
    - (5) Other lipid storage diseases



Gaucher's disease, lymphosarcoma, or Bock's sarcoma. Splenectomy frequently restores a normal peripheral blood picture without affecting the underlying disorder.

### ***Congenital Absence of the Spleen***

Agenesis of the spleen usually occurs in combination with malformation of the heart, commonly atrioventricular communis and partial transposition of the abdominal viscera.<sup>33-35</sup> Rarely is there an absence of an associated anomaly.<sup>34, 36</sup> In either case a presumptive diagnosis of agenesis of the spleen can be made from the peripheral blood by the presence of normoblastemia and Howell-Jolly bodies and Heinz bodies in the erythrocytes. The diagnostic value of Heinz bodies (particles of denatured hemoglobin) has been emphasized by their presence in 10 per cent of the red cells in the peripheral blood of mature newborn infants with agenesis of the spleen.<sup>36</sup>

### ***Primary Splenic Neutropenia***

Varying degrees of leukopenia and granulocytopenia accompany most conditions with splenomegaly in the form of a secondary hypersplenism. Primary splenic neutropenia, however, consists strictly of neutropenia with normal granulocytes and platelets, often a palpable spleen but without evidence of an underlying disease. Primary splenic neutropenia is a rare disease in childhood and should be differentiated from chronic or periodic neutropenia. The symptoms and signs in both conditions overlap. There are frequent bouts of fever, sore throat, ulcerative lesions of the gums, mouth, tonsils, vulva, and vagina. The leukocyte count varies between 1,000 and 3,000 per cubic millimeter with granulocytes varying from 0 to 20 per cent.<sup>1</sup> In the typical patient symptoms are completely relieved by splenectomy.

### ***Felty's Syndrome***

Felty's syndrome occurs in adults. It consists of neutropenia, splenomegaly, and chronic rheumatoid arthritis and usually responds to splenectomy.<sup>36</sup>

### ***Primary Splenic Panhematopenia***

Although primary splenic panhematopenia occurs frequently in children with splenomegaly as a manifestation of secondary hypersplenism, the primary disease is rare. The case of a 14-year-old girl with the primary disease was described by Doan and Wright, with complaints of weakness, pallor, unexplained fever, skin disorders, and anorexia, accompanied by pancytopenia, a hyperplastic marrow, a slightly palpable nontender spleen, and no adenopathy. Splenectomy was followed by a hematologic as well as clinical recovery. As contrasted with aplastic anemia in which pancytopenia also exists, the bone marrow in patients with primary splenic panhematopenia is hyperplastic and each cellular element is present in normal or increased numbers.

A few of the better known disorders in infancy and childhood, nonleukemic or neoplastic in origin, are associated with a greatly enlarged spleen. Out

- A Acute
  - (1) Septicemias
  - (2) Salmonella infections
  - (3) Brucellosis
  - (4) Infectious mononucleosis
- B Chronic
  - (1) Malaria
  - (2) Kala azar
  - (3) Trypanosomiasis and other parasitic infections
- C Subacute bacterial endocarditis
- D Tuberculosis
- E Sarcoidosis
- F Syphilis
- 5 Neoplasms and cysts
  - A Lymphosarcoma
  - B Hodgkin's disease
  - C Follicular lymphoma
  - D Reticulum cell sarcoma
  - E Hemangioma and lymphangioma
  - F Dermoids
- 6 Miscellaneous
  - A Lupus erythematosus
  - B Rheumatoid arthritis

### **Hypersplenism**

The spleen exerts a regulatory influence on the control of blood formation and in the delivery of cellular elements from the bone marrow. This function is greatly exaggerated when the spleen becomes hyperactive a condition which is termed hypersplenism and implies an exaggeration of inhibitory and destructive activities of the spleen. Hypersplenism represents a functional and not an anatomic change. Inherent in this concept is the reduction of one or more cellular elements in the peripheral blood with compensatory hyperplasia in the bone marrow of the corresponding cells, the presence of an enlarged spleen and the expectation that the peripheral blood picture will return to normal or nearly normal by splenectomy. The blood disturbances dependent upon hypersplenism have been attributed to either selective sequestration and increased destruction of formed cell elements in the enlarged spleen or to an inhibitory influence upon a normal or hyperactive bone marrow by a hormonal mechanism.<sup>18, 19</sup> By inhibitory action it is understood that the growth and maturation of various cells are prevented or else their delivery from the marrow to the blood is blocked. In either case whether by increased destruction or inhibition the removal of an abnormally functioning spleen has been shown to restore normal blood levels with varying degrees of success. Hypersplenism therefore is associated with neutropenia, thrombocytopenia or anemia either singly or in combination by splenomegaly and a normal or hypercellular marrow.

Hypersplenism may be primary when there is no obvious cause for the depletion of blood cell types as there is in patients with splenic neutropenia, splenic pancytopenia or pancytopenia and idiopathic thrombocytopenic purpura. It is secondary in patients with splenomegaly in combination with well defined disorders such as Banti's syndrome, Hodgkin's disease, chronic leukemia

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biliary cirrhosis and portal hypertension in childhood. It stems from mechanical obstruction of the bile ductules by inspissated secretions.<sup>2</sup> Cirrhosis of the liver which accounts for 70 per cent of cases of portal hypertension in the adult is rare in children. In children extrahepatic lesions are the more common and are due to congenital malformations such as congenital stenosis or atresia, aneurysm of the splenic artery and distortions of the portal vein extending into the liver. Other causes are thrombosis resulting from thrombophlebitis caused by omphalitis or generalized infection in early life involving splenic or portal veins or both, cavernomatous transformation of the portal vein and compression from pancreatic fibrosis and tumors especially of the pancreas.

**Collateral Circulation.** In the patient with chronic portal hypertension a collateral circulation which tends to lower the pressure in the portal system develops between the portal and systemic veins. These collaterals are located at the lower end of the esophagus and the upper end of the stomach in the umbilicus and in the rectum. Collateral vascular channels are sometimes seen in the abdominal wall. Of the greatest clinical significance are the collateral routes beneath the mucous membranes at the cardioesophageal junction which give rise to esophageal varices. The latter are thin walled and are likely to rupture when exposed to trauma resulting in massive and at times fatal hemorrhage. The hemorrhoidal varices represent connections between the portal system and systemic veins through the middle hemorrhoidal vessels and constitute a source of hemorrhage.<sup>3</sup> Although it is known that bleeding from ruptured esophageal varices can occasionally occur in the absence of associated portal hypertension, patients with a high portal venous pressure and massive bleeding from esophageal varices have been described in whom neither intrahepatic nor extrahepatic portal vein obstruction was found. Esophageal varices have been observed also in patients with extensive intrahepatic Hodgkin's disease.<sup>29</sup>

**Pathology.** The long standing back pressure on the venous sinuses of the spleen eventually results in hemorrhage with fibrotic and regenerative changes within this organ resulting in a characteristic fibrotic spleen.<sup>30</sup> The pathologic histology of the spleen has been described as consisting of periarterial hemorrhages developing into areas of perarterial fibrosis, siderotic nodules and dilated venous sinuses with thickening of the reticulum of the wall giving a collagen staining reaction.<sup>40</sup>

**Clinical Features.** The onset is insidious usually with an unexplained enlargement of the spleen. The most common manifestations are fatigability, pallor, splenomegaly, hematemesis or melena. Portal hypertension should be suspected in children of any age even in the first year of life when hematemesis or melena is associated with an enlarged spleen. In an infant who had had melena and hematemesis from 3 months of age, splenoportography at 14 months revealed anomalies of the portal system. In patients with fully developed chronic congestive splenomegaly the liver is palpable and the spleen is massive and in over half of the patients hematemesis has occurred. Less often hemorrhoids are a source of bleeding. The bulk of these features are attributable to the rupture of varices in strategic locations. Epistaxis and early bruising are noted in a small number of patients.

standing examples are chronic congestive splenomegaly (Banti's syndrome) and Letterer-Siwe disease. Gaucher's disease and Niemann-Pick disease. The latter are diseases of the reticuloendothelial systems.

### ***Chronic Congestive Splenomegaly (Banti's Syndrome Portal Hypertension, Splenic Anemia)***

Chronic congestive splenomegaly is characterized by enlargement of the spleen, progressive anemia, leukopenia, often thrombocytopenia, gastrointestinal hemorrhage due to portal hypertension and in later stages cirrhosis of the liver and ascites.

**Etiology and Pathogenesis.** As originally described by Banti in 1894<sup>6</sup> the disease progresses in three stages. The first is that of splenic enlargement and increasing anemia; the second, enlargement of the liver and jaundice; and terminally, cirrhosis of the liver, gastrointestinal hemorrhage and ascites. The sequence is initiated by a toxin elaborated by an enlarged spleen which acts locally and is also carried to the liver and other tissues and organs. The causative agent being lodged primarily in the arteriole of the Malpighian corpuscle produces a thickening of the surrounding reticulum while maintaining a gland-like structure. Banti designated this pathologic picture as "fibroadenia." It has since been shown<sup>49</sup> that "fibroadenia" was indeed a peripheral fibrosis representing a nonspecific manifestation of passive congestion and an end result of hemorrhage around the splenic arterioles. More recently the concept of Banti's disease has been changed from a homogeneous entity originating in the spleen to one of congestion and enlargement of the spleen resulting from high portal venous pressure.<sup>50</sup> Congestive splenomegaly has therefore replaced the term Banti's disease.

The veins which form the portal system are the portal, the superior and inferior mesenteric and the splenic veins and their tributaries. The portal vein itself is formed by the union of the superior mesenteric and splenic veins. Unlike other veins the portal vein ends like an artery, breaking up into numerous small channels which ultimately terminate in capillaries in the substance of the liver. The portal vein has no valves and carries about three-fourths of the circulation of the liver, whereas the hepatic artery carries oxygen and supplies the other fourth of the circulation. Both vessels have a common outlet in the hepatic vein which empties into the inferior vena cava. It is important to bear in mind that back pressure in the valveless portal system does not possess the anatomic barriers found in the peripheral veins.<sup>50</sup>

The location of the obstruction in the portal system determines the type of portal hypertension. If obstruction is within the liver it is classified as intrahepatic; if outside the liver parenchyma it is classified as extrahepatic. Normally the venous pressure in the portal system ranges from 140 to 220 mm. of saline solution or from 60 to 140 mm. of water.<sup>51</sup> In the adult the portal pressure is normally below 225 mm. of water so that readings above 250 mm. can be regarded as abnormally high.<sup>50</sup>

The commonest cause of portal obstruction is cirrhosis of the liver resulting from congenital diseases, infiltrations, hepatitis, schistosomiasis and neoplasms. Fibrocystic disease of the pancreas is one of the more important causes of

Newer techniques have led to an elucidation of the factors concerned with the pathogenesis of portal hypertension and the possible identification of the site of obstruction. Appraisal of liver function, manometric measurement of portal pressures and the use of portal venography contribute to the accuracy of diagnosis and facilitate surgical intervention for decompression of the portal area.<sup>67</sup> In portal venography the portal venous system is rendered opaque by contrast medium and then visualized by serial roentgen studies. At present percutaneous splenoportography is in common use and provides important data with regard to size and confirmation of the vessels and thus aids in the selection of the optimal type of shunt.<sup>68</sup>

Splenorenal and portacaval anastomoses are the two types of shunts in common use and the choice of one or the other depends largely upon the caliber of the vessels available for the procedure. Splenectomy might be considered preferable in children with moderate or marked enlargement of the spleen, especially when pancytopenia exists. Although blood values would be restored to normal or nearly normal, it is now realized that once the spleen is removed the splenic vein is no longer available for anastomosis should it later become necessary. *Splenectomy without a venovenous shunt is indicated only in patients with lesions obstructing the splenic hilum.*<sup>6</sup> The prognosis is best in those children with a normal liver and with obstruction located in the splenic vein.

Splenectomy with a splenorenal shunt is usually recommended in patients with marked splenomegaly, hypersplenism, large caliber splenic veins or an obliterated portal vein resulting from cavernomatous transformation or aplasia of the portal vein. A portacaval shunt is advocated with small caliber splenic veins or cirrhosis of the liver. Because of the progressive nature of the disease and the likelihood that a fair percentage of splenorenal shunts will tend to close spontaneously, there has been a tendency to decompress the portal system by an immediate portacaval shunt, especially in the small child. Should the latter procedure eventually prove ineffective, the now larger splenic vein will be available for anastomosis. It is of interest that a salutary effect on the hematologic features of hypersplenism has been observed after a portacaval shunt with a reversal of the pancytopenia and without splenectomy. In general it is practically impossible to establish a good shunt in a child under 2½ to 3 years of age.

Anemia due to hemorrhage responds to iron therapy. Blood transfusions are given for values of 8 gm per 100 ml or less, but the hemoglobin concentration need not be elevated to maximal levels. Esophageal bleeding remains a constant source of concern and methods for its control by tamponade with esophageal balloons have proved of questionable value.

## DISEASES OF THE RETICULOENDOTHELIAL SYSTEM

The reticuloendothelioses constitute a group of disorders of unknown etiology, having in common hyperplasia of cellular elements of the reticuloendothelial system. Reticulum cells and histiocytes undergo proliferation principally in the spleen, liver, bone marrow, lymph nodes and to some extent in other tissues and organs. These disorders have been subject to varied classifications but

**Laboratory Data** Normochromic and hypochromic anemia and leukopenia with or without thrombocytopenia are usually noted. With increasing size of the spleen marked reduction in the number of leukocytes occurs ranging usually between 1 500 and 4 000 white blood cells per cubic millimeter with a pre dominance of lymphocytes. Leukocytosis accompanies severe hemorrhage and when blood loss is repeated a hypochromic microcytic anemia results. In the presence of portal cirrhosis the red blood cell survival may be shortened and indirect serum bilirubin may be elevated.<sup>4</sup> The mechanism postulated for hemolysis is a high portal venous pressure and probably a major degree of congestive splenomegaly with hemostasis. Microcytosis<sup>56</sup> and an increase in the mean corpuscular hemoglobin value have been noted in patients with long standing disease and cirrhosis of the liver.<sup>61</sup> In patients with marked thrombocytopenia prolonged bleeding time and occasionally increased bruising follows but this association is inconstant. The coagulation time is usually normal. The bone marrow reveals either a normal pattern despite leukopenia and anemia or a hyperplasia involving megakaryocytes and myeloid and erythroid elements.

**Diagnosis** The combination of massive enlargement of the spleen, a palpable liver and pancytopenia often presents a difficult diagnostic problem. A history of hematemesis or melena is of course highly suggestive of portal hypertension. After barium is swallowed esophageal varices are visualized in only about 40 per cent of patients.<sup>6</sup> In patients suspected to have chronic congestive splenomegaly the use of percutaneous splenoportal venography has been helpful in diagnosis. Before a definite diagnosis can be made other conditions associated with splenomegaly, leukopenia and pancytopenia must be eliminated. These include Gaucher's disease, Niemann-Pick disease, nonlipid reticuloendotheliosis (Letterer-Siwe disease) and infiltrations of the bone marrow with leukemic or neoplastic cells.

**Course and Prognosis** The course depends upon the degree and site of obstruction and the effectiveness of a remedial operation in relieving the excessive portal venous pressure and retarding liver damage. The control of gastrointestinal hemorrhage from ruptured varices and eradication of the obstruction are important factors in projecting the outcome. The presence of ascites and persistent anorexia are of serious prognostic import.

**Treatment** Adequate treatment depends upon the discovery of the site and nature of the portal obstruction. Extrahepatic portal hypertension may be differentiated from that which is secondary to liver cirrhosis<sup>7</sup> and is suggested by the following: a history of omphalitis or severe bacterial infection during early infancy which is followed by an uneventful course until signs of portal hypertension appear; an essentially negative and benign history prior to the onset of signs of portal hypertension such as hematemesis and/or splenomegaly; an absence of jaundice or other signs of liver disease prior to the onset of symptoms; and normal liver function tests. In contrast portal hypertension secondary to cirrhosis of the liver is suggested by positive liver function tests, evidence of a previous history of jaundice or liver enlargement, hepatomegaly and tenderness of the liver which is prominent. Hepatic enlargement, ascites and other evidences of liver failure tend to appear prior to portal hypertension.



Newer techniques have led to an elucidation of the factors concerned with the pathogenesis of portal hypertension and the possible identification of the site of obstruction. Appraisal of liver function, manometric measurement of portal pressures and the use of portal venography contribute to the accuracy of diagnosis and facilitate surgical intervention for decompression of the portal area.<sup>6</sup> In portal venography the portal venous system is rendered opaque by contrast medium and then visualized by serial roentgen studies. At present percutaneous splenoportography is in common use and provides important data with regard to size and confirmation of the vessels and thus aids in the selection of the optimal type of shunt.<sup>9</sup>

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are now separated on the basis of presence or absence of distinctive intracellular lipids. Gaucher's disease and Niemann Pick disease are the prominent members of the storage disease group and Letterer-Siwe disease (nonlipid reticuloendotheliosis) and Hand-Schüller-Christian disease and eosinophilic granuloma constitute the inflammatory group. The difficulty of classification is exemplified by Hand-Schüller-Christian disease in which the proliferating histiocytes initially contain very little cholesterol but later accumulate enough to give the appearance of foam cells.

### *Gaucher's Disease*

Gaucher's disease is an uncommon hereditary metabolic disorder characterized by the storage of cerasins and other cerebroside in the reticuloendothelial system. It has been most often observed in Jewish families but cases have been described in many nationalities over the world. The disease occurs as an acute infantile type and a chronic or adult type.

**Pathology and Pathogenesis.** At least three substances have been isolated from tissues containing Gaucher cells: a galactosiderocerebroside (cerasin), a glucosiderocerebroside, and a water-soluble glycolipid polycerebroside.<sup>6</sup> The pathogenesis of the disease has not been fully established. It has been ascribed to a primary disturbance of intermediary lipid metabolism causing an elevation of cerebroside in the serum with later storage in reticuloendothelial cells or to a primary disturbance in the reticulum cells causing increased synthesis and storage of cerebroside.<sup>4</sup> Postmortem examination reveals Gaucher cells in the spleen, liver, lymph nodes, bone marrow, lungs, and other organs. The disease is diagnosed by the demonstration of these cells in areas where they proliferate, the most accessible of which is the bone marrow. Splenic puncture also serves as a useful means of demonstrating the source of Gaucher cells. These cells are large and distinctive, 20 to 80 microns in diameter, round or oval, and possess one or more small dense nuclei eccentrically located. The cytoplasm has an opaque, wrinkled tissue paper appearance due to the presence of fine wavy fibrils running parallel to the long axis of the cell. The cytoplasm occupies the major part of the Gaucher cell and stains slightly gray or bluish.

**Clinical Features.** The clinical features of the acute infantile and chronic forms of Gaucher's disease are discussed separately.

**Acute Infantile Form.** The infant may appear normal at birth and for the first weeks and months of life but soon undergoes mental and physical retardation and deterioration. Splenomegaly followed by enlargement of the liver contributes to prominence of the abdomen, which is further exaggerated by wasting of the extremities. Severe neurologic symptoms and signs characterize the infantile form of the disease. Generalized hypertonia, opisthotonus, and rigidity, dysphagia, laryngeal spasm, cyanosis, severe cough due in part to pulmonary infiltration with Gaucher cells, and fever dominate the clinical picture. Death follows before the age of 2 years.<sup>1</sup> The cerebral changes observed at postmortem examination indicate chronic disease of the ganglion cells progressing to sclerosis and complete destruction. Rarely, typical Gaucher cells are found in the intracerebral vascular spaces.<sup>8</sup>

**Chronic Form** The chronic form has an insidious onset (starting in childhood or at any age thereafter) most commonly with splenomegaly followed soon after by liver enlargement. Lymphadenopathy is not a conspicuous feature. The skin reveals a yellow or patchy brown pigmentation which is particularly prominent on exposed parts of the body—face, neck, hands and legs. Pinguiculae



Fig. 41. A Photomicrograph of nest of Gaucher cells in smear of bone marrow ( $\times 900$ )  
 B A single Gaucher cell ( $\times 1500$ ) from the smear shown in A. Note crinkled fibrillar appearance of cytoplasm and relatively small slightly eccentric nucleus.

of the conjunctiva are rare in children and common in adults. They consist of a brownish yellow wedge shaped thickening of the subconjunctival tissue with bases situated close to the corneal margins and apices pointed to the inner and outer canthi. Infiltration with Gaucher cells causes pulmonary and bone involvement. Pain in the legs occasionally accompanied by swelling of adjacent

joints is due to bony infiltration by Gaucher cells. The roentgenogram shows diffuse or localized destructive and productive changes often producing a characteristic deformity in the lower femora. This consists of a widening of the lower halves and thinning and flaring of the cortices giving an Erlenmeyer flask appearance.<sup>21</sup> This feature is reminiscent of the swollen appearance in similar areas in patients with severe Cooley's anemia. Pathologic fractures due to marked osteoporosis and replacement by Gaucher's cells may occur.

**Blood.** The anemia is usually of a mild or moderate normochromic and normocytic type. Leukopenia with relative lymphocytosis and slight to marked thrombocytopenia may be present. Thrombocytopenia with hemorrhage may be sufficiently severe to require splenectomy. The complete pancytopenic blood picture of hypersplenic disorders may ultimately develop. Serum lipid and cholesterol levels are normal.

**Heredity.** The disease has been noted in siblings in a parent and child both with the full clinical picture of Gaucher's disease<sup>22</sup> and in asymptomatic parents of typically affected children.<sup>23</sup> In this respect the parent can be regarded as a carrier. The mode of inheritance varies with different groups, the majority of cases being caused by an autosomal recessive gene.<sup>23</sup>

**Course and Treatment.** There is no cure for Gaucher's disease and in children beyond infancy the disease may either progress or remain chronic. The lives of those who survive to adolescence are prolonged for many years. Most adults die of intercurrent diseases rather than of Gaucher's disease. Splenectomy is usually effective in relieving the development of a massive spleen and in reversing the severe pancytopenia.<sup>23</sup>

### **Niemann Pick Disease**

Niemann Pick disease is a rare hereditary disease resembling in its clinical features the infantile type of Gaucher's disease.

**Clinical Features.** The onset may date from birth or may occur after the first six months of life. Progressive physical and mental deterioration is accompanied by massive and equal enlargement of the liver and spleen. In contrast to patients with Gaucher's disease the spleen is larger than the liver. A brownish yellow pigmentation occurs especially in the parts exposed to the light. In an appreciable number of patients estimated as high as 60 per cent<sup>24</sup> a cherry red spot appears in the macula corresponding to that seen in patients with amaurotic familial idiocy (Tay Sachs disease). Nervous system involvement is manifested by spasticity, blindness and deafness; the patient finally lapses into a state of apathy and idiocy. With the patient profoundly emaciated death usually occurs before the third year of life. Although the bone marrow is infiltrated with foam cells survival is not sufficiently prolonged for changes to be conspicuously apparent on x-ray examination.

Chronic forms of the disease are described extending to adolescence<sup>16</sup> and in cases reported in two brothers death occurred at 29 and 33 years of age respectively.<sup>24</sup> In patients who have survived beyond infancy extensive pulmonary infiltrations are observed on roentgenographic examination. In a 19-year-old boy under observation from early childhood with the typical Niemann

Pick cells in the bone marrow marked hepatosplenomegaly and pulmonary infiltration normal mental and physical growth took place

**Pathology and Pathogenesis** The striking features at autopsy are involvement of the liver spleen lungs bone marrow and lymph nodes and the replacement of reticular cells and histiocytes by the foam or Niemann Pick cells The nervous system is almost invariably affected Degenerative changes take place in the ganglion cells The large neurons are distended or ballooned with loss of their usual triangular or pyramidal shape Usually there are lipid deposits in the ganglion and neuroglia cells <sup>8</sup> Often there is a paucity of nerve cells as if many had disintegrated <sup>10</sup>



Fig 4. Photomicrograph of bone marrow smear of patient with Niemann Pick disease Note group of typical cells—their large size relatively small round or oval nuclei and foam droplets giving a honeycomb appearance of cytoplasm

The storage material in the foam cells which accumulates in the viscera of patients with Niemann Pick disease consists largely of phospholipids chiefly lecithin and sphingomyelin These foam cells which characterize the disease are readily available for examination by bone marrow aspiration These are large more or less rounded occasionally oval or polyhedral cells averaging 15 to 90 microns in diameter<sup>16</sup> and containing one or two nuclei often eccentrically placed with loosely arranged chromatin material The abundant cytoplasm is filled with highly refractile lipid droplets giving a weblike honeycombed or foamy appearance Unlike the Gaucher's cells the foam cells of the disease are readily detected in the counting chamber and can be separated from the megakaryocytes <sup>3</sup> (see Fig 42)

Blood Vacuoles appear in the cytoplasm of the circulating lymphocytes and monocytes but no definite histochemical studies on such cells are known

The vacuoles are discrete unstained and round and vary in number from 1 or 2 per cent to 15 or 20 per cent <sup>10</sup>

In the older patient anisocytosis and poikilocytosis with many oval cells are noted. A mild or moderate microcytic anemia is found. White blood cells vary between slight leukocytosis and moderate leukopenia. Blood cholesterol either is not increased or is slightly elevated.

**Heredity** There is a striking predilection for Jews which is more marked than in Gaucher's disease. The occurrence among siblings is well known. Tay Sachs disease has been noted among relatives of patients with Niemann Pick disease.

**Treatment** There is no effective treatment. Splenectomy has been carried out but except for the relief of anemia and mild evidences of hypersplenism this procedure has not altered the course of the disease <sup>10</sup>

### ***Letterer Siwe Disease, Hand Schüller Christian Disease, and Eosinophilic Granuloma***

There is considerable evidence that these three syndromes are members of a closely related group of disorders whose underlying pathology is an inflammatory histiocytosis. Each has distinctive clinicopathologic features which are currently regarded as different expressions of the same basic disorder <sup>3</sup>. Of these Letterer Siwe disease is acute and malignant eosinophilic granuloma is the most benign and Hand Schüller Christian disease occupies an intermediate position. Overlapping occurs to the extent that some histologic changes will be found in each entity which are similar to those of the other two <sup>5</sup>.

### ***Letterer Siwe Disease (Nonlipid Reticuloendotheliosis)***

Letterer Siwe disease is an acute nonfamilial disease appearing before the age of 2 years with a rapidly fatal course within weeks or months of the onset <sup>69 70 81</sup>. The etiology is unknown. Often the disease is ushered in as an acute infection at times there is no relation to infection. Irritability, malaise, diarrhea, and development of a skin eruption which may date from birth are early features. A hemorrhagic tendency manifested by petechiae and purpura combines with a yellow scaly greasy seborrheic eruption which is accentuated over the scalp and trunk <sup>31 40 9</sup>. A purulent otitis media, hepatosplenomegaly, moderately enlarged and tender lymph nodes, abdominal enlargement and fever characterize the disease in the infant. Bronchitis and bronchopneumonia are common. Circular areas of rarefaction in the calvaria and cystlike defects in the lower ends of the femora and upper ends of the tibiae have been described <sup>15</sup>. Pulmonary involvement due to histiocytic infiltration appears on the roentgenogram as diffuse irregularly nodular involvement. Progression to multiple lung cysts have been described <sup>38</sup>.

**Pathology** The spleen, liver, lymph nodes, skin, lungs, and bone marrow are heavily infiltrated by sheets of histiocytes, granulomatous nodules, and multinucleated giant cells. There is no lipid storage except in occasional patients in whom there are foam cells containing cholesterol. The rapid course

of the disease precludes progression to the histologic picture of Hand Schuller Christian disease.

**Diagnosis** A hemorrhagic tendency, eczematoid eruption enlargement of the liver spleen and lymph nodes roentgenographic evidence of bony defects and progressive anemia in a young infant are highly suggestive of Letterer Siwe disease. The characteristic eruption is not invariable in the infant nor does it appear in the older child in which case diagnosis depends upon roentgenographic findings and the pathologic evidence of a generalized hyperplasia of histiocytes. Biopsy of skin or lymph nodes reveals evidence of proliferating histiocytes and occasional foam cells.

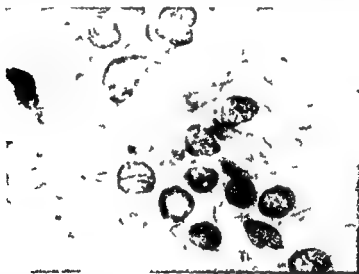


Fig. 43 Touch preparation from the skin lesions ( $\times 725$ ) of a 20-month old patient with nonlipid histiocytosis (Letterer Siwe disease). The large mononuclear cells resemble those seen in affected lymph nodes. (From Moore T. D.: A Simple Technique for the Diagnosis of Non Lipid Histiocytosis. *Pediatrics* 19: 438, 1957.)

Bone marrow aspiration often demonstrates increased numbers of histiocytes. These are irregularly round, oval or polyhedral cells with protoplasmic projections containing ingested particles, red blood cells and leukocytes. The nucleus is round, oval or kidney shaped and is usually eccentrically placed.<sup>51</sup> It should be remembered that occasional phagocytic histiocytes of this type are seen in bone marrow smears of patients with many pathologic states. Skin scrapings show cells similar to the proliferating reticulum cells in the lymph nodes, spleen, liver and bone marrow and serve as a diagnostic aid.<sup>46</sup>

**Blood** Severe and progressive anemia is a prominent feature. The red cells are normochromic and normocytic and the white blood cell count ranges from leukopenic to moderate leukocytosis with lymphocytosis or a normal differential count. Hemohistiocytes have been identified in the peripheral blood.<sup>50</sup> Despite the hemorrhagic nature of the disease the platelets may be normal or only

slightly decreased in numbers. Occasionally there is marked thrombocytopenia. Pancytopenia and a hyperplastic bone marrow are occasionally noted. The etiology of the hematologic changes is by no means clear. A hemolytic mechanism has been considered on the basis of persistent reticulocytosis, nucleated red cells in the peripheral smears, slightly elevated serum bilirubin, and erythroid hyperplasia of the bone marrow.<sup>10</sup>

**Treatment and Course.** Supportive therapy, transfusions, radiation, and antibiotics<sup>10</sup> have been employed. Improvement with ACTH and steroids<sup>8</sup> has been reported, but this form of therapy awaits further trial. The disease is usually fatal in infants. The chronic case in the older patient may represent a transitional form between Letterer-Siwe disease and Hand-Schüller-Christian disease.

### *Hand-Schüller-Christian Disease*

Hand-Schüller-Christian disease is a constitutional disorder of metabolism characterized by a classic triad consisting of defects of membranous bones, exophthalmos, and diabetes insipidus. These features and associated phenomena result from the localized accumulation of histiocytes containing cholesterol and its esters, giving the appearance of foam cells (xanthomas).

**Clinical Features.** The onset is insidious, occurring most often in children before the age of 7 years and often after an infection. The disease is ushered in by varied signs and symptoms indicative of underlying accumulations of histiocytes and foam cells. Ulceration of the mouth, loose teeth, swollen gums, dwarfism, yellow to brownish-colored plaques and nodules of the skin, and chronic otitis media with mastoid involvement are characteristic findings.<sup>11-13</sup> Papular eczematoid lesions, which resemble the dermatitis of Letterer-Siwe disease, start on the scalp and spread over the face and trunk. Xanthomatous lesions are uncommon. When present, they consist of pinpoint to pea-sized nodules from the deeper layer of the skin and are covered by normal epidermis.<sup>14</sup>

**Lesions in the skeleton** are part of a generalized skeletal disease and consist of numerous punched-out areas of bone resulting in defects of irregular size and producing a geographic pattern. The defects are filled by a yellow granular tissue originating in the dura or periosteum. Although the skull defects are most prominent, the facial bones, pelvis, ribs, scapula, and spine may be subject to granulomatous formation, giving rise to localized areas of bone destruction which can be visualized on roentgenograms.<sup>15</sup> Pathologic fractures result from cysts in the long bones and punched-out areas. Diabetes insipidus is due to xanthomatous involvement of the region of the hypophysis and base of the brain. Exophthalmos follows destruction of the orbit and replacement by abnormal tissues. Visceral involvement leads to serious complications. Bronch involvement is unusual. Infiltration of the lungs, suggesting the picture of pneumoconiosis on the roentgenogram, leads ultimately to marked fibrosis, cor pulmonale, and right heart failure. Involvement of the liver, spleen, and lymph nodes is less common than involvement of other systems, although it may be a predominant feature. Growth and sexual development are retarded.

**Pathology.** On microscopic examination the most notable histologic feature is the presence of foam cells lodged in different tissues, giving a yellow coloration



The fully developed foam cell is large pale round or ovoid and 20 to 40 microns in diameter having abundant cytoplasm and one or two small nuclei. In the fresh state it is loaded with small droplets of fatlike material.<sup>42</sup> Biopsies show a hyperplasia of histiocytic foam cells or an admixture with granulomatous tissue rich in eosinophils. This close pathologic relationship is confirmed by cases demonstrating the transition from eosinophilic granuloma to Hand Schuller Christian disease.<sup>3, 43, 44</sup>

**Diagnosis** Although membranous bone defects, exophthalmos, and diabetes insipidus are the cardinal features of Hand Schuller Christian disease, they are not always present together, especially in the initial stages. In one series they were present in combination in only three of twenty-nine patients.<sup>4</sup> Examination of a single biopsy specimen does not permit unequivocal classification of any given case. In such a case the diagnosis and subsequent course must be deferred until the disease becomes generalized. With an incomplete picture there may be confusion with isolated bone cysts, multiple myeloma, Ewing's sarcoma, and metastatic neuroblastoma. Usually there is no anemia; if it does occur it is mild. A severe progressive anemia is indicative of serious involvement of blood-forming organs. Serum cholesterol is normal.

**Course** The course is chronic and protracted, but spontaneous remissions occur. When the onset is early in life, the course is more likely to be severe than when it comes later. Visceral and neurologic lesions presage a fulminating course.<sup>45</sup> Of the series of twenty-nine cases cited previously, the mortality was 13 per cent.<sup>4</sup> The prognosis is always doubtful, and a true estimate still awaits the results of more extensive experience with modern therapy.

**Treatment** Roentgen ray therapy results in definite improvement, being limited to the lesion toward which it is directed. In soft tissue masses and enlarged lymph nodes a response is obtained within two to three weeks, and in bone defects a response is obtained after three to four months. It is the quickest available method for healing bone lesions and for relieving exophthalmos.<sup>46</sup> Steroid therapy, intravenous nitrogen mustard, and the antifolates such as methopterin are useful adjuvants. Polydipsia and polyuria of diabetes insipidus are easily controlled by injection or insufflation of Pitressin.<sup>44</sup>

### *Eosinophilic Granuloma*

Eosinophilic granuloma, the main feature of which is the presence of single or multiple skeletal lesions,<sup>1</sup> is regarded as the most benign member of the reticuloendothelial group.<sup>4</sup> It occurs predominantly in infants, children, and young adults. Any bone in the body may be involved, but there appears to be a predilection for the bones of the skull, vertebrae, extremities, and pelvis. Visceral involvement is rare. There is no hepatosplenomegaly or lymph node enlargement. Usually there is little or no systemic evidence of illness. Symptoms, when present, are referable to the local lesion and consist of mild fever, local pains, swelling, and redness. The foci of destruction, as well as demarcated areas of rarefaction, are represented in the roentgenograms. Expansion of the lesions in the medullary cavity of the long bones results in erosion of the cortex and spontaneous fractures. In other instances cyst formation in the long bones

is associated with regional cortical thickening<sup>12</sup>. The bony defects showing a punched out appearance are similar to those of Hand Schuller Christian disease. Pulmonary nodular infiltrations of eosinophilic granuloma may coexist with<sup>13</sup> and without<sup>10, 14</sup> associated skeletal lesions.

Histologically the lesions consist of granulomatous inflammatory collections interspersed among which are polymorphonuclear leukocytes and accumulations of eosinophilic leukocytes. Multinuclear giant cells and foam cells occasionally are present. The number of eosinophilic leukocytes in the peripheral blood is usually not increased. Transitional forms between Hand Schuller Christian disease and eosinophilic granuloma occur. The lesions respond well to surgical excision or simple curettage performed at the time of biopsy. Roentgen ray therapy is also effective. Spontaneous cures have been observed. The prognosis is favorable but must be guarded because of the possibility of skeletal lesions appearing elsewhere.

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## Blood Coagulation

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An abnormal tendency to bleed is the most obvious indication of a disturbance of hemostasis. Such an event calls for an investigation of possible defects in one or more of the components which normally maintain hemostasis. A description of these factors and their interaction will be reviewed as a background to discussion of the hemorrhagic disorders.

The arrest of hemorrhage is a complex process involving vascular integrity, platelets, and a group of specific circulating proteins which are active in blood coagulation. The sequence from the initial response to skin and blood vessel injury to the formation of a clot requires the proper functioning and coordination of a series of vascular and clotting mechanisms. These are so closely integrated that it has been stated that a single abnormality of one of the hemostatic mechanisms does not necessarily result in bleeding if all the others are normal.<sup>109</sup>

### NORMAL HEMOSTATIC MECHANISMS

**Vascular Factors** When a vessel is injured sufficiently to permit the escape of blood, an immediate reflex contraction occurs. In the smallest venules and capillaries hemostasis is accomplished by direct adhesion of endothelial surfaces.<sup>9</sup> Since the media of veins contain less muscle than those of arteries, vasoconstriction is less marked. Venous hemostasis depends mainly on the accumulation of platelets at the edges of the vessel wall which eventually occludes the vessel. In the small arteries and arterioles vasoconstriction permits adherence and subsequent clumping of platelets by contracting the smooth muscle. The formation of large amorphous hyaline clumps derived from the platelets (viscous metamorphosis) seals off the injured area. Contraction of the vessel is reinforced by this disintegration of platelets and by the release of a powerful vasoconstrictor serotonin (5 hydroxytryptamine).<sup>1-3</sup>

In the small arteries the platelet mass is reinforced by fibrin resulting from blood coagulation during vasoconstriction. Platelets and precipitated fibrin threads form a hemostatic plug which fills the lumen of the vessel. The clot subsequently retracts and is partially digested and organized and the vessel is recanalized. Hemorrhage from a larger artery is controlled with difficulty until the blood pressure drops sufficiently within the vessel for a blood clot to form.

Extravascular factors such as subcutaneous tissue muscle bone and skin contribute to the arrest of the hemorrhage by presenting a firm surface so that the local accumulation of blood compresses the affected blood vessel. Ascorbic acid is regarded as a necessary factor for the synthesis of intercellular cement substance in the capillaries which unites the individual endothelial cells—hence its use in all defined bleeding syndromes due to increased vascular fragility. Spontaneous bleeding occurs with loss or changes in the cement substance. The continuity of capillaries is further strengthened by the adherence of platelets to this substance. Vitamin P is apparently also involved in some unknown manner in maintaining capillary permeability but its administration therapeutically (rutin hesperidin and bioflavonoids) has been disappointing.

**Role of Platelets** Platelets normally are small granular disk shaped non nucleated bodies measuring 2 to 5 microns in diameter. In patients with disease they may vary in size from fine particles to masses of agranular cytoplasm about twice the size of the normal platelet. Platelets arise by budding from the cytoplasm of megakaryocytes in the bone marrow. In the earlier forms of megakaryocytes the megakaryoblast and promegakaryocyte the cytoplasm stains deep blue is nongranular and shows no evidence of platelet formation. These primitive cells are increased in numbers in patients with thrombocytopenic purpura. In the mature megakaryocyte the cytoplasm is abundant and basophilic and contains numerous azurophilic granules. Masses of mature platelets often adhere to the periphery of the cells. The nuclei are large and are joined together in an irregularly lobulated ring.

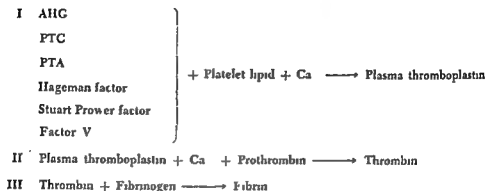
Many properties and activities associated with coagulation and hemostasis have been identified with platelet function. Some of these have been mentioned in connection with vascular factors. Platelets tend to adhere to injured blood vessels check the formation of petechiae and extravasations of blood in spontaneous hemorrhage promote clot retraction and enhance vasoconstriction. It has been demonstrated that the platelet is a highly adsorptive structure and that many of the factors are merely carried on or are adherent to their surface.<sup>59</sup> The plasma accelerator globulin (labile factor) for instance which functions in blood coagulation is adsorbed by the platelet.<sup>60</sup> In addition to liberating a vasoconstrictor substance platelets contribute a number of factors to coagulation. Platelets accelerate the conversion of prothrombin to thrombin (platelet factor 1) accelerate the conversion of fibrinogen to fibrin (platelet factor 2) participate in the formation of thromboplastin (platelet factor 3) and neutralize the action of heparin (platelet factor 4). An example of the relation of platelet factors to coagulation is observed in patients with severe thrombocytopenia in whom the marked reduction of platelets (less than 20 per cent of normal) retards prothrombin conversion to thrombin and is responsible for poor clot retraction.<sup>118</sup>

The platelet factor participating in thromboplastin generation (factor 3) is a phospholipid. Numerous phosphatides among them brain<sup>119</sup> and soybean cephalins<sup>120</sup> have been substituted for platelets in a variety of test systems to measure thromboplastin formation.

**Blood Coagulation Mechanism** In the classical coagulation theory of Morawitz thromboplastin originates from two sources the tissues and platelets

When tissues are injured preformed thromboplastin (thrombokinase) is liberated which in the presence of calcium converts prothrombin to thrombin. Acting as an enzyme, thrombin then converts fibrinogen to fibrin. These studies provided a basis for subsequent investigations dealing with coagulation.

#### PLASMA THROMBOPLASTIN SYSTEM



#### TISSUE THROMBOPLASTIN SYSTEM

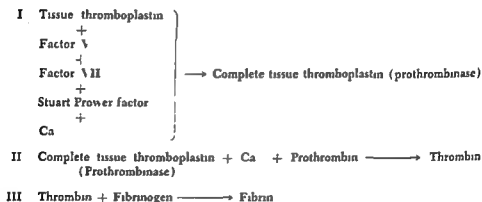


Fig. 44 Schematic outline of the coagulation mechanism

It is now known that platelets contain no preformed thromboplastin but that they do interact with plasma factors to form plasma thromboplastin. In the presence of calcium, plasma thromboplastin converts prothrombin to thrombin without the aid of accessory factors. In contrast, tissue extract (incomplete thromboplastin) performs the same function but only after it has been activated by specific factors in the blood. This form of thromboplastin is termed prothrombinase. Plasma (intrinsic) thromboplastin evolved during the coagulation of normal blood is therefore differentiated from the extrinsic thromboplastin formed by the reaction of tissue extracts with other factors. The intrinsic and extrinsic mechanisms operate concurrently, the latter being more immediately mobilized for hemostasis in injured areas.

**Coagulation Factors** Coagulation of blood free from tissue juice has been arbitrarily divided into three stages: the elaboration of plasma thromboplastin



the conversion of prothrombin to thrombin and the interaction of thrombin with fibrinogen to form the fibrin clot. These main stages are assisted by accelerating and inhibitory mechanisms. The precise mode of action of several of the factors and their relative importance in each phase of coagulation are still being elucidated and in many ways are still controversial.

Many of the coagulation factors in plasma and serum to be discussed are described under numerous synonyms and abbreviations some of which are given in Table 17.

*Table 17 Coagulation Factors and Their Designations*

| Coagulation Factors              | Synonyms and Abbreviations                                                                                                                         |
|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Antihemophilic globulin          | AHIC AHIF<br>Thromboplastinogen<br>Factor VIII                                                                                                     |
| Plasma thromboplastin component  | PTC<br>Christmas factor<br>Factor IX                                                                                                               |
| Plasma thromboplastin antecedent | PTA                                                                                                                                                |
| Factor V                         | Labile factor<br>Plasma accelerator factor<br>Plasma Ac globulin to serum Ac globulin<br>Proaccelerin to accelurin                                 |
| Factor VII                       | Stable factor<br>Proconvertin to convertin<br>Plasma precursor to serum prothrombin conversion accelerator (SPCA)<br>Prothrombin conversion factor |

**PHASE 1** The first phase of blood coagulation is concerned with the elaboration of plasma thromboplastin free from tissue juice. Contact with a foreign surface and injury to a blood vessel initiate coagulation by the disintegration of platelets and the release of a lipoid thromboplastic factor. Many cofactors react with platelets. Several cofactors may be regarded as being of major importance in coagulation: antihemophilic globulin (AHG)<sup>110</sup> plasma thromboplastin component (PTC)<sup>61, 111</sup> plasma thromboplastin antecedent (PTA)<sup>69, 100</sup> factor V (labile factor) and the Stuart Prower factor.<sup>6, 7, 8, 9, 111</sup> Reference is also made to other participating factors: factor X<sup>112</sup> and the Hageman factor<sup>9, 98</sup>. Calcium is essential. These soluble plasma clotting factors with the exception of fibrinogen are present in small amounts. Nevertheless for the synthesis of active

At a meeting for the standardization of nomenclature of blood clotting factors<sup>113</sup> it was decided that the Stuart Prower factor was similar in many respects to factor X as described by Kollmer.<sup>114</sup> The committee therefore suggested the Roman X to designate the Stuart Prower factor. This change does not deny the possibility of a liver factor formerly regarded as factor X. It is deficient in patients with liver disease.

intrinsic thromboplastin it is obligatory that they be available in adequate concentration

The importance of PTA in coagulation has been questioned by some<sup>1</sup> whereas others regard it as a major factor. In our experience a substantial decrease of PTA results in a hemophilia like clinical picture similar in many respects to AHG and PTC deficiency.

In patients with Hageman factor deficiency<sup>20</sup> coagulation time is prolonged but affected persons have no symptoms and have been operated upon without excessive bleeding. The clot promoting effect of glass upon normal human plasma requires the presence of the Hageman factor.<sup>20</sup> This factor a surface activation factor probably aids in the initiation of the clotting process. A 9 year-old boy with a deficiency of the Hageman factor was completely free of hemorrhagic symptoms despite a prolonged clotting time.<sup>20</sup> The content of Hageman factor may be low in many infants during the newborn period. Unlike factor VII the Stuart Prower factor and plasma thromboplastin component (PTC) which do not reach normal levels until 6 weeks of age or more the concentration of Hageman factor is normal by 10 to 14 days of life.<sup>20</sup>

The Stuart Prower factor is necessary for both the first and second phases of blood coagulation. It is utilized for the production of thromboplastin as well as for the conversion of prothrombin to thrombin. A deficiency of the Stuart Prower factor retards the thromboplastin generation test and prolongs the prothrombin time. This factor is gradually assuming increasing clinical importance with the observation that it may be deficient in congenital and acquired liver disease and in hemorrhagic disease of the newborn infant.<sup>24</sup>

Antihemophilic globulin is associated with the globulin of the fibrinogen fraction of the plasma. protein II is labile when stored and is completely utilized in the process of clotting whereas PTC and PTA are stable when stored and are not consumed in this process. These properties are of practical importance in laboratory testing and in the selection of therapeutic agents.

**PHASE 2** The second phase of blood coagulation is concerned with the conversion of prothrombin to thrombin. In this process intrinsic thromboplastin generated in the first phase reacts with prothrombin and calcium to form thrombin. Tissue juice liberated by trauma reacts with the accessory factors (factor V factor VII Stuart Prower factor and calcium) to form an extrinsic thromboplastin or prothrombinase.<sup>25, 61, 6</sup> The interaction of these two substances is conceived as follows: active plasma thromboplastin and calcium initially convert prothrombin to thrombin slowly in small amounts. At this point prothrombinase formed in the presence of calcium increases the velocity of the prothrombin conversion and is responsible for the rapid phase of thrombin formation.<sup>67</sup> Factor V and factor VII once activated are referred to as the accelerators of prothrombin conversion. They are not precursors of thrombin but are accelerators which influence the speed at which thrombin is formed in the presence of tissue extracts.<sup>1</sup>

Although no authentic case of a hemorrhagic disease due to a deficiency of calcium has been reported, calcium appears to play a role in the first two phases of coagulation. This activity takes place in its diffusible ionized form. The anti-coagulant effect of citrate is mediated through suppression of calcium ionization whereas in the case of oxalate free calcium is precipitated.

Prothrombin is a glycoprotein which is stable when stored and which is utilized in excess of 25 per cent during the coagulation process. A sufficient intake of vitamin K is necessary for normal synthesis of prothrombin provided liver function is normal.

Factor V deteriorates in oxalated plasma and therefore is referred to as the labile factor. It is also called *ac globulin* and *pro accelerator*. Factor VII is also known as stable factor, *pro convertin* and serum prothrombin conversion accelerator (SPCA).

Factor V is consumed during clotting; stable factor is not. A deficiency in either factor V or Stuart Prower factor results in a hemorrhagic state due to decreased formation of prothrombinase with subsequent inadequate thrombin formation. Factor V and Stuart Prower factor are also necessary for the formation of intrinsic thromboplastin in the first phase of coagulation. Factor VII is not necessary for thromboplastin generation in coagulating blood in contrast to the Stuart Prower factor or for conversion of prothrombin to thrombin by the intrinsic or plasma thromboplastin. Factor VII represents one of the factors that is involved in the reaction with tissue extract to form extrinsic thromboplastin which directly activates prothrombin.

**PHASE 3** In the final phase of coagulation fibrinogen is converted to fibrin through the action of thrombin liberated in phase 2. Fibrinogen with a molecular weight of 350,000 is an unstable soluble protein formed in the liver. In the plasma fibrinogen reaches a concentration of 250 to 400 mg per 100 ml. The final step, clot retraction, appears to be controlled by intact platelets. Disorders characterized by inadequate fibrin formation include congenital afibrinogenemia and congenital and acquired hypofibrinogenemia.

**Dynamics of Coagulation** Blood clotting should be thought of as a dynamic process moving in the direction of fibrin formation. It should be emphasized that the sequence of events outlined here in three distinct phases is based upon *in vitro* studies. They serve a convenient and useful purpose in the clinical and laboratory approach to the patient with a hemorrhagic disorder. Blood coagulation *in vivo* probably does not occur in such sharply demarcated series of steps but in a slow initial phase and a succeeding accelerated one.

As soon as a small amount of thrombin is found, platelets begin to be labilized and to disintegrate. This activates thromboplastin which in turn converts prothrombin to thrombin. From this point the generation of thrombin takes place with increasing velocity, reaching explosive force. The rapid phase has been referred to as a chain reaction or an autocatalytic mechanism and assures the presence of enough thrombin<sup>30</sup> at the site of injury. The initial slow phase parallels the period from the disintegration of platelets to the formation of small amounts of thrombin. The succeeding accelerated phase extends from steps involving the rapid evolution of thrombin to the conversion of fibrinogen to fibrin. Thrombin thus represents the key substance in the chain reaction of blood coagulation. These changes correspond roughly to blood coagulation in a test tube in which the blood remains fluid for several minutes (slow phase) and then clots solidly in a short fraction of time (accelerated phase).

**Natural Inhibitors of Coagulation** With the forces favoring coagulability of the blood, there are corresponding opposing physiologic inhibitors and anti-coagulants which maintain its fluidity. Maintaining the continuity of the vascular endothelium constitutes one of the most important factors preventing the formation of thromboplastin. *In vivo* the formation of thrombin is counteracted by natural inhibitors such as antithrombin and heparin. The latter is extracted from the liver and is also present in the granules of the mast cells. The adsorption of thrombin by fibrin may also be regarded as a neutralizer of thrombin.

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Classic hemophilia occurs sporadically in children without a previous family history in about 25 to 30 per cent of patients either because the disease has gone unrecognized or because it is the end result of a mutation with the de novo appearance of either hemophilic males or female carriers.<sup>41</sup>

**Clinical Aspects** The disease is characterized by recurrent episodes of bleeding from various parts of the body occurring either spontaneously or following slight injury. The tendency toward prolonged hemorrhage is observed in the first days of life either from the umbilical cord which is rare or from circumcision which is common. The bleeding from the latter source may be trivial or moderate or may require transfusion because of its persistence. Hemorrhage occurs almost invariably in the first year of life with the onset of walking commonly following injuries of the nose and mouth especially lacerations of the lips tongue frenulum of the upper lip and gums. Excessive bleeding during inoculations or during eruption or loss of deciduous teeth suggests hemophilia.

Petechiae are rare. Subcutaneous and intramuscular hemorrhages are common. A significant amount of blood may be lost into the large muscles such as those of the thighs and gluteal regions. A person with hemophilia may bleed from the mucous membranes into the pleural or peritoneal cavities the gastrointestinal tract solid viscera or central nervous system. Hematuria is common and often persistent but responds to vigorous therapy. Epistaxis is uncommon in childhood. Hemorrhage into the retroperitoneal region into the mesentery and into the iliopsoas may simulate acute appendicitis. Abdominal pain presents complex problems in differential diagnosis. More often the signs and symptoms are due to bleeding into the intestine wall and peritoneal cavity than to inflammation although mild leukocytosis can be present in either case.

In addition to blood loss tissue hemorrhages may cause serious pressure effects. Hematomas in strategic areas such as those in the extremities may obstruct circulation. Bleeding into the tissues of the mouth neck and thorax may seriously interfere with respiration producing asphyxia.

Spontaneous hemorrhages frequently are cyclic with short episodes recurring at approximately three to eight week intervals despite the constancy of the deficiency. With equal deficiency of antihemophilic globulin children show variations in clinical severity.

Bleeding after circumcision in one newborn infant with hemophilia and no bleeding in another with equally severe hemophilia must be due to the amount of tissue juices expressed during the procedure. The cord blood from a newborn infant with classic hemophilia was found to be devoid of antihemophilic globulin<sup>42</sup> indicating the lack of passage of a plasma factor from the mother.

**Hemarthrosis** The most characteristic site of hemorrhage is the joint cavities usually of the ankles knees and especially the elbows. The shoulders wrists and hips may also be involved. Acute hemarthroses occur suddenly with severe pain swelling heat tenderness and limitation of motion. Although early hemorrhages are readily absorbed the recurrence of bleeding eventually leads to extensive damage and thickening of synovial membranes destruction of articular sur-

## DISORDERS DUE TO A DEFICIENCY OF FACTORS REQUIRED FOR THROMBOPLASTIN FORMATION (PHASE 1 OF COAGULATION)

*General Consideration of the Hemophilias* Until recent studies demonstrated its manifold nature, the term hemophilia was applied to a single disorder—a deficiency of the antihemophilic globulin (AHG) known to be essential for clotting and hemostasis. The discovery of the additional thromboplastin precursors plasma thromboplastin component (PTC) and plasma thromboplastin antecedent (PTA) led to the realization that hemophilia was not an isolated condition but a group of entities with a similar symptomatology.<sup>22</sup> The pathogenesis of each of the three members could then be attributed to a congenital inability to produce a specific thromboplastin precursor. As in patients with classic hemophilia, those with other hemophilias also demonstrate a bleeding tendency from childhood and give a history of other bleeders in the family. From the standpoint of management and specific therapy, the separation of the hemophilias from one another has proved to be necessary.

In due course methods were developed for the differentiation of each type of defect within the hemophilia group. An extension of these and other techniques revealed also that many cases of clotting disorders which had heretofore been classified as hemophilia were due to deficiencies in other phases of coagulation, principally in the prothrombin complex. In all types and grades of hemophilia, the platelet count, bleeding time, clot retraction, prothrombin time, and fibrinogen concentrations are normal.

A feature of major importance in the diagnosis of hemophilia is the variation in its severity. The mild form of the disease is most deceptive since its manifestations emerge suddenly and unexpectedly after surgical procedures, especially dental extraction or tonsillectomy. The degree of clinical severity in mildly affected patients is correlated to some extent with the percentage of antihemophilic globulin in the plasma.

### *Classic Hemophilia (Hemophilia A, AHG Deficiency)*

*Hereditary Aspects* Classic hemophilia is a severe congenital disorder consisting of a hereditary defect in blood thromboplastin formation due to a congenital deficiency of antihemophilic globulin. The condition is inherited as a sex-linked recessive mendelian trait with transmission to affected males by asymptomatic carrier females who possess a normal content of antihemophilic globulin. The daughter of an affected male is capable of transmitting the trait to half of her sons who will have the disease and half of her daughters who will be carriers. Hemophilic carriers as a group show significantly lower than normal plasma antihemophilic globulin concentrations, but a considerable overlap with the normal range may exist.<sup>23,24</sup> Abnormal results with available tests for blood coagulation were obtained in a substantial number of carriers with hemophilia A (AHG deficiency) and hemophilia B (PTC deficiency).<sup>25,26</sup> In rare instances classic hemophilia occurs in the female as a product of the marriage between a hemophilic male and a carrier female.<sup>27</sup>

given if the patient is anemic. In the use of plasma a knowledge of its properties is important. AHG is labile; its life span in the circulation is twelve to twenty-four hours and its half life is four to six hours so that 50 per cent of the initial level will remain after four hours, 25 per cent after eight hours etc.

Fresh plasma that is withdrawn a few hours after collection of blood frozen rapidly at  $-20^{\circ}\text{C}$  and stored in a frozen state maintains its activity for variable periods of time. According to one estimate fresh frozen plasma kept at  $-20^{\circ}\text{C}$  loses roughly half its strength in one month after which time the remaining AHG tends to be stable.<sup>68</sup> According to another study<sup>9</sup> up to half of the donor's AHG is lost during centrifugation for separation of the plasma; the half life of the remainder in the wet frozen state is about sixteen weeks. In contrast AHG activity in bank blood and plasma shows progressive deterioration with about 30 to 60 per cent of the initial AHG remaining after three weeks storage.<sup>69</sup>

The plasma from freshly collected blood may be lyophilized and reconstituted with saline solution before use. AHG in lyophilized plasma is also remarkably stable for as long as one year. One commercial preparation of frozen lyophilized plasma (Antihemophilic Plasma<sup>®</sup>) contained AHG levels averaging 72 per cent of fresh normal plasma.<sup>72</sup> This preparation is of value in traveling and in areas in which fresh frozen plasma is not readily available.

Studies in our laboratory have confirmed the findings of Brinkhous and co-workers<sup>9</sup> with regard to the AHG concentration required for hemostasis in patients with hemophilia. They found that the minimum hemostatic level of AHG may be as low as 5 per cent of normal although some data suggested that it might be 15 to 20 per cent of normal or higher. A constant level of AHG between 10 and 20 per cent is realized by administering plasma in a dose of 10 ml per kilogram of body weight initially and 5 ml per kilogram every hour.<sup>101</sup> It has been frequently observed that active hemorrhage will persist in patients with hemophilia after inadequate amounts of plasma or blood are given although the clotting time becomes normal.

The accompanying schedules provide guides for treatment of hemorrhage in patients with various clinical conditions and for preparation of patients for surgical procedures. Amounts in excess of those given in the schedules may be necessary because the plasma AHG values of normal persons vary widely—in one estimate ranging from about 60 to 175 per cent of the mean.<sup>9</sup> In treatment of patients with hemophilia the initial dose of plasma should be injected fairly rapidly (within a period of forty-five minutes to one hour) in order to supply the bleeding area with adequate amounts of AHG to control hemostasis.

*For emergency surgery and bleeding into dangerous areas*

First day—10 ml per kilogram of body weight initially

5 ml per kilogram of body weight every four hours

Second day—10 ml per kilogram of body weight every eight hours

Third day—10 ml per kilogram of body weight every twelve hours for remainder of week

faces and erosion of bone with chronic inflammatory lesions contractures and permanent crippling

**Management** Treatment of the patient with severe hemophilia requires detailed attention to many aspects of the disease. As an initial step it is essential to determine whether the deficiency is due to the AHC factor or PTC factor and to assay the degree of deficiency by laboratory tests. The parents must be informed of the physical and emotional adjustments that will have to be made by both the patient and the family. Of major importance is the recognition of the special aptitudes of the child so that necessary training will be given for a protected occupation in adult life.

The individual management of the patient with hemophilia entails a knowledge of treatment of local bleeding sites and acute internal bleeding episodes of preparation before dental extraction minor operations emergency surgery and of orthopedic care.

**Treatment of Bleeding** Treatment of bleeding consists of local measures and transfusions of whole blood or fresh frozen or lyophilized plasma.

**Local Measures** Treatment consists of the application of a suitable coagulant and local pressure at the bleeding site. For open wounds, powdered thrombin and local packing with absorbable packs such as a fibrin form oxidized cellulose (Oxycel) or gelatin sponge (Gelfoam) saturated in a solution of topical thrombin and Adrenalin followed by firm pressure are valuable hemostatic devices. When it is not possible to apply digital pressure to an accessible bleeding site a useful procedure is to spray a mixture of Adrenalin and thrombin on the local area after it has been freed of excess clot.<sup>33</sup> Thrombin is not to be given intravenously. Cauterization is always contraindicated. Suturing a wound should be avoided since it may provoke more serious local bleeding and tissue necrosis. Local measures are not a substitute for transfusion or replacement therapy with frozen plasma especially if the bleeding is located in a potentially dangerous area.

**Transfusion of Whole Blood or Plasma** For the patient who has suffered extensive blood loss immediate transfusion with whole blood or packed cells is necessary. Fresh whole blood is preferable if available. In many patients it is unnecessary to bring the hemoglobin level to maximum concentrations; levels of 10 to 11 gm per 100 ml are adequate. Short of severe anemia the basic objective in treatment of the patient in the acute phase or in preparation before a surgical procedure to prevent bleeding is to inject into the circulation a sufficient amount of antihemophilic globulin to raise its concentration to a level which will assure effective hemostasis. In Christmas (PTC) disease the same objective is sought.

Potent concentrates of AHC from human or animal sources (bovine or pig globulin) have been successfully employed. However the animal AHC material has been difficult to sterilize and because it is a foreign protein it is potentially antigenic with the hazard of anaphylactic reactions.<sup>34</sup>

**USE OF PLASMA—FRESH FROZEN AND LYOPHILIZED** Transfusion of type specific fresh frozen plasma is the treatment of choice. A whole blood transfusion is



urine output should be measured. However in all patients with excessive bleeding within cavities the initial increments of the calculated dose of plasma should be given rapidly and the remaining amount given according to the clinical condition of the patient. Douglas has recently recommended the use of approximately 1 liter (or 10 to 15 ml per kilogram of body weight) of fresh frozen plasma given by rapid drip within forty five minutes to one hour and repeated every six to twelve hours to maintain a level of a required 14 per cent of AHG. In patients with severe bleeding 10 to 15 ml = preferably given every four to six hours.

The maintenance of adequate and continuous concentrations of AHG is especially important in patients with deep tissue hemorrhages in whom important structures may be involved. Such situations are encountered in the preparation for and during emergency surgery, especially of an abdominal nature and in patients with central nervous system bleeding. Fatalities in patients with hemophilia are often due to ill advised surgery. Hemorrhage into the tissues of the tongue and throat requires active treatment with plasma and prompt pharyngeal or laryngeal intubation to prevent fatal asphyxia.<sup>9</sup> Hemothorax usually occurs spontaneously and produces serious respiratory and cardiac embarrassment. Aspiration is usually urgent and should be preceded by the rapid administration of fresh frozen plasma in adequate quantities.

The use of prophylactic plasma transfusions or plasma fractions at periodic intervals carries the hazard of a refractory state due to circulating anticoagulants.<sup>10</sup> Management of the refractory state is most unsatisfactory. Withholding of blood or plasma is recommended with the hope that the anticoagulant will disappear. Treatment however is to be resumed when active bleeding recurs. Steroids have been given for their suppressive action on antibody formation and except for bringing about a possible improvement in patients with cutaneous hemorrhages have been largely ineffective. However they are worthy of further and more extensive trial.

More important in prophylaxis is the protection of the infant and young child against trauma by carefully selecting toys, padding the crib, removing potential sources of injury from furniture when the child begins to walk and supervising play activities. With advancing age and greater understanding accidents are decreased.

**Treatment of Hemarthroses.** Hemarthroses are particularly troublesome and tend to recur. Acute joint bleeding is to be treated promptly and vigorously for three to four days with plasma as suggested in the schedules for treatment. Reassurance, analgesics, plasma therapy, bed rest and elevation with slight flexion of the joint surrounded by ice packs constitute initial treatment (plastic ice bags should be kept immediately available in the refrigerator). An elastic compression bandage is useful. Following arrest of hemorrhage orthopedic supervision is necessary for conserving maximum function and preventing deformities.

With the control of bleeding cautious active motion within painless areas and massage are instituted until the former range of movement is obtained.<sup>24</sup> Active motion favors absorption of residual blood and prevents contracture of capsular and pericapsular tissues.<sup>27</sup> Instillations of hyaluronidase into the affected

*Dental extraction*

- 10 ml per kilogram of body weight one half hour before extraction
- 5 ml per kilogram of body weight one hour after extraction and 10 ml per kilogram of body weight every twelve hours
- 10 ml per kilogram of body weight daily thereafter for four days

*Hemarthrosis*

- 10 ml per kilogram of body weight once a day for at least two days
- Skip third day
- 10 ml per kilogram of body weight on fourth day

In lacerations of the tongue lip or frenulum in an infant intensive replacement therapy will be necessary for the first two days and more moderate amounts of plasma are given for the remainder of a week to ensure the formation of a firm clot. For the small infant an entire unit of fresh frozen plasma (125 ml) is injected by drip every six hours for the first forty eight hours instead of a fraction every four hours then tapering off to two units daily. Sedation is necessary to prevent premature dislodging of the clot. The vein is kept open by a slow drip of an electrolyte solution between plasma injections. In an infant it is essential to guard against overloading the circulation with fluid by checking on urinary output.

Occasionally vigorous treatment over a prolonged period with plasma will lead to progressive edema and abdominal pain without cessation of bleeding from such sites as an extracted tooth or from the kidneys. We have found it expedient in this emergency to stop plasma infusions while maintaining local hemostasis. After a rest period transfusions with packed cells whole blood or plasma are reintituted depending upon the hemoglobin level.

Biggs and Macfarlane<sup>8</sup> classified severely affected patients with spontaneous bleeding as having less than 1 per cent of AHG mildly affected patients as having more than 5 per cent and the variably affected group as having between 0 and 5 per cent. Concerning treatment they state that all patients requiring transfusions had less than 30 per cent of AHG that if the AHG level can be raised to between 15 and 30 per cent postoperative bleeding will be less excessive and that if the AHG level can be raised to more than 30 per cent postoperative bleeding may be prevented. Brinkhous pointed out that injected AHG is quickly redistributed between vascular and extravascular compartments. Once equilibrium is established between these compartments the rate of loss of AHG from the plasma may then be an indication of its biologic half life.

In the patient with hematuria rigid adherence to the schedule outlined for active bleeding is required until bleeding is completely controlled. During this period the patient must be maintained on complete bed rest.

Severe hematuria in a 15 year-old boy was controlled within a five day period by vigorous treatment according to the preceding schedule. Each two unit dose of fresh frozen plasma (a total of 250 to 300 ml) was given within an hour in order to maintain the necessary concentration of AHG in the circulation. Packed red cells were transfused when the hemoglobin concentration dropped below 9 gm per 100 ml. Between treatments the vein was kept open with an electrolyte solution given by very slow drip. Complete bed rest was mandatory while the urine contained significant numbers of red cells. A similar successful result was obtained one year later when hematuria recurred. Because of increasing weight with age the possibility of overloading the circulation by fluid must be kept in mind and

in the bones due to hemorrhages into adjacent joints<sup>23</sup> Hemorrhages into the spongiosa of the shafts and epiphyses produce cystic areas of rarefaction Juxta articular cysts irregular in size and distribution are an outstanding feature seen on the roentgenogram of the patient with moderate hemophilic arthropathy<sup>6</sup>

Marginal bony defects of the epiphyses and erosions of joint margins are noted In the joints incomplete resorption of blood and retained blood clots result in deformities disability and infrequently ankylosis Repeated hemorrhages into joint spaces may lead to accelerated maturation and hypertrophy of the adjacent epiphyses probably resulting from local hyperemia<sup>5</sup> (see Fig 45) With limitation of motion generalized decalcification is noted in the bones adjacent to the affected joint

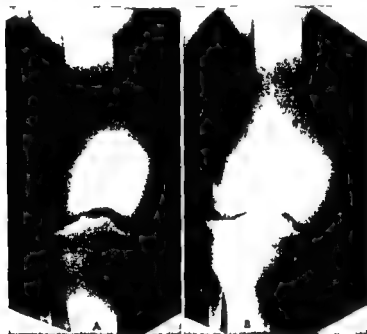


Fig. 45 Roentgenograms of knees of a patient with hemophilia (AHG deficiency) A, Normal epiphyses of right knee B Note hypertrophy of epiphysis of femur and tibia of left knee due to local hyperemia from recurrent hemarthrosis

**Prognosis** Deaths from exsanguination following surgical procedures or severe trauma have become less common with the judicious administration of fresh plasma or blood The protection offered the infant and young child obscures the true nature of the disease which becomes apparent as the child grows older and is exposed to trauma of various kinds Hemarthrosis resulting from frequently repeated hemorrhages eventually results in crippling and disability It has been stated that the best laboratory guide to prognosis is the determination of the blood concentrations of AHG Patients with no detectable

joint have been advocated as a means of inducing rapid restoration of motion without pain in hemarthrosis.<sup>2</sup> More extensive experience is needed to evaluate this form of treatment as well as the aspiration of joints which is the practice in some clinics.

Experience in our clinic has demonstrated the value of prednisone (20 mg daily in divided doses) in reducing pain. It is given for a week or ten days after joint bleeding has been controlled by plasma and in patients with milder hemarthrosis it is given without the use of plasma. A combination of aspirin and small amounts of steroids has been particularly efficacious. Where a joint has been repeatedly affected rehabilitation with traction casts and braces is necessary.<sup>6</sup>

**Mild Hemophilia** One of the significant observations made concerning hemophilia as a result of correlating clinical manifestations with laboratory findings is the variation in its severity. On the basis of assay it appears that severely affected persons have no measurable amounts of antihemophilic globulin in the plasma and in the most mildly affected patients it ranges from 5 to 20 per cent and in a smaller number it is less than 5 per cent.<sup>3</sup> In general AHG levels above 5 per cent occur in mildly affected patients.<sup>3</sup> Under strain of the hemostatic mechanism such as occurs postoperatively, occasionally a seemingly well person with an AHG value between 30 and 45 per cent may suffer from transient bleeding. It has been stated that each person appears to have a constant and characteristic level of this clotting factor. It should again be emphasized that patients with mild deficiencies may not manifest hemorrhage until they sustain a sizeable injury or after such common surgical procedures as tonsillectomy or dental extraction.

**Diagnosis** An abnormal bleeding tendency, particularly hemarthroses dating from infancy occurring also in other members of the family and being limited to male members with evidence of sex-linked inheritance suggests hemophilia. In patients with severe grades of hemophilia the marked prolongation of whole blood clotting time is a characteristic feature. In patients with mild types of disease the coagulation time may be slightly prolonged or even normal since as little as 1 per cent of AHG is sufficient to produce a normal clotting time. The prothrombin consumption test and the thromboplastin generation tests are more sensitive tests of an AHG deficiency with amounts below 5 per cent and 20 per cent respectively in the plasma.

The thromboplastin generation test by identifying the deficient factor will separate hemophilia B due to PTC defect from classic hemophilia which it closely resembles clinically. In the type of pseudohemophilia (von Willebrand's disease) in which there is a combined vascular defect and AHG deficiency epistaxis and a prolonged bleeding time characterize the former and hemarthrosis and a normal bleeding time the latter.

Mixing tests provide simple diagnostic leads. The three types of plasma correct unrelated hemophilias but not those with the corresponding defect.

**Roentgenographic Findings** In the early stages of hemophilia x-ray findings are nonspecific and consist of swelling and distention of the joint. Skeletal lesions result from bleeding directly into the bones or from secondary changes

In still another group an abnormality of platelets is an added feature<sup>24</sup> A reduction in the plasma concentration of antihemophilic globulin was observed in the members of four families suffering from von Willebrand's disease<sup>21b</sup> Such persons were clinically affected.

### *Plasma Thromboplastin Component Deficiency (Hemophilia B, Christmas Disease)*

Plasma thromboplastin component deficiency<sup>6, 12, 103</sup> often designated as Christmas disease<sup>104</sup> is a hemophilia like disease due to a deficiency of plasma thromboplastin component (PTC) one of the necessary thromboplastin precursors It is inherited as a sex linked recessive and therefore is confined to males and transmitted by a carrier female as is classic hemophilia As in patients with AHG deficiency there is no family history in about 25 to 30 per cent of the patients

*Clinical and Laboratory Features* PTC deficiency constitutes about 15 per cent of all hemophilias and is clinically indistinguishable from classic hemophilia It also exists as a mild disease Hemarthroses are common The clotting time is prolonged (normal in patients with the mild form of the disease) and prothrombin consumption is abnormal (due to inadequate thromboplastin formation) but the actual defect is diagnosed by the thromboplastin generation test (located in the serum component as contrasted with the plasma component in patients with classic hemophilia) In newborn infants with hemorrhagic disease and in patients with liver disease PTC deficiency is often found in combination with prothrombin and stable factor deficiencies An assay of the PTC factor<sup>5</sup> demonstrated that a 30 per cent concentration of the factor is sufficient to protect against ordinary every day stresses and as much as 60 per cent is needed to stop bleeding from major wounds These figures correspond to 5 to 10 per cent and 30 to 40 per cent AHG respectively under the same conditions in patients with classic hemophilia Of seven carrier females and one probable carrier four had abnormally low PTC levels

*Treatment* Accuracy of diagnosis especially differentiation from AHG deficiency is important The stability of PTC when stored permits the use of banked blood or plasma Fresh plasma therefore is not essential as it is in treatment of hemophilia On the other hand because of the greater availability of fresh frozen plasma it is usually employed Since the critical level of PTC for effective hemostasis is similar to that of AHG in patients with hemophilia A comparable amounts of plasma are given The intervals between administration may be prolonged to forty eight to seventy two hours because of the longer survival of PTC than AHG in the blood stream Details of management as to dental extraction preparation for surgery and treatment of hemarthroses are similar to those for patients with classic hemophilia Specific inhibitors of PTC as of AHG have been known to develop<sup>105</sup> probably as a result of repeated transfusions of whole blood and plasma

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 Aft
 and tion 3-year-old boy named Christmas who was among the first patients in whom this
 diagnosed¹⁰⁶

AHG are usually severely affected and those with more than 5 per cent are almost invariably mildly affected.³

Vascular Hemophilia (von Willebrand's Disease, Pseudohemophilia B)

Vascular hemophilia is characterized by a moderate to severe AHG deficiency combined with a capillary defect causing defective prothrombin consumption and prolonged bleeding time.^{8,33, 91,10, 10, 114} It was originally described by von Willebrand¹⁰ as pseudohemophilia. The main features consist of a markedly prolonged bleeding time, normal clotting time, normal platelet count, normal clot retraction, and occurrence in both males and females.

With the advent of newer techniques, investigation showed that von Willebrand's disease comprised two groups: patients with a vascular (capillary) defect plus AHG deficiency (vascular hemophilia¹⁰⁴ pseudohemophilia B¹⁰¹) and patients with a vascular defect with a normal coagulation status (pseudohemophilia A). The morphologic vascular abnormalities consisted of coiled, tortuous capillaries in the nail beds and in the venules of the bulbar conjunctivae. These abnormalities were present with or without associated deficiency of antihemophilic globulin and may account for the characteristic prolonged bleeding time in the von Willebrand syndrome.

Although severe intracranial hemorrhage can occur, the most common symptom in either group of patients regardless of associated AHG deficiency is severe and spontaneous epistaxis. Also common to both groups is bleeding from the tongue, gums, and teeth following extraction or loss of deciduous teeth. Excessive bruising on slight trauma is also present. The outstanding laboratory finding is a prolonged bleeding time. The clotting time, prothrombin time, platelet count, and clot retractions are normal. The capillary fragility is only occasionally abnormal. The coagulation abnormality, deficiency of AHG if severe enough, may be reflected in the impaired prothrombin consumption and defective thromboplastin generation tests. The amount of antihemophilic globulin in patients with vascular hemophilia is in excess of that found in those with classic hemophilia and corresponds to concentrations in patients with mild hemophilia (about 1 per cent of AHG).

The presence of severe epistaxis, which characterizes vascular hemophilia, separates this disease from mild hemophilia in which the bleeding time is normal. The disease is familial, occurs in both sexes, is probably hereditary, and is transmitted as a simple dominant. Administration of fresh plasma corrects the coagulation defect and has proved of value in treatment before and after dental extraction. Splenectomy is definitely contraindicated. Vitamin P, vitamin C, rutin, and steroids have proved ineffective in controlling epistaxis and other manifestations of the abnormal vascular component.

The fact that transfusion of normal plasma¹¹ or a plasma fraction^{84, 116} occasionally corrects the bleeding time as well as the coagulation time suggests the possibility that vascular dysfunction may result from a deficiency of a plasma vascular factor required for normal vasoconstriction, which is different from AHG.

fore consists of prothrombin and labile stable and Stuart Prower factors. Each deficiency will be included in this discussion of phase 2 coagulation although labile and Stuart Prower factors are also involved in the first phase of coagulation with blood thromboplastin formation.

Deficiencies of the factors of the prothrombin complex may be congenital in origin or may be acquired in various disease states. Members of the prothrombin complex are synthesized in the liver so that deficiency of one or all of the factors is found in patients with liver disease. Combined deficiencies of the four components are most commonly found in patients with parenchymal liver disease such as infectious hepatitis and cirrhosis. Since prothrombin and stable factor deficiencies are usually found in combination it is rare to find a disease condition in which one occurs to the exclusion of the other. Deficiency of both factors may be further associated with plasma thromboplastin component (PTC) deficiency in patients with liver disease and in newborn infants with hemorrhagic disease.⁴ Deficiency in the latter may be mainly due to liver immaturity.

Hypoprothrombinemia (Vitamin K and Prothrombin Deficiency)

Vitamin K is a dietary principle required for the normal clotting of blood. In chicks fed fat free diets Dam⁵ observed the development of hemorrhages in various tissues and noted further that it was associated with a longer clotting time of the blood. It was soon determined that the delay in clotting resulted from a decrease in prothrombin and that the hemorrhagic tendency resulting from this deficiency could be corrected by feeding the chicks substances containing an active principle, vitamin K. Prothrombin is synthesized by the liver from vitamin K and absorbed by the intestine but the mechanism by which synthesis is achieved is still unknown. Deficiencies of vitamin K regardless of cause are now known to result in a depletion of both prothrombin and stable factors but not in labile factor. The administration of vitamin K corrects both prothrombin and stable factor deficiencies.

Hypoprothrombinemia combined with stable factor deficiency occurs in various states in which vitamin K is reduced. Since natural vitamin K is fat soluble bile is required for its absorption. A deficiency is very likely to occur whenever bile is prevented from entering the intestinal tract as occurs in patients with biliary obstruction and in those with hepatic disease due to toxic and infectious agents since the reactions of vitamin K in prothrombin synthesis are impaired. Deficiencies occur in the presence of faulty intestinal absorption such as occurs in patients with celiac disease, diarrhea, gastrointestinal malformation and steatorrhea. Green vegetables and products of intestinal bacteria are among the richest sources of vitamin K. Hypoprothrombinemia due to a deficient intake of this vitamin is rare because prothrombin is capable of being elaborated from the small amounts of this vitamin commonly found in foods and also produced by the intestinal flora. In newborn infants hypoprothrombinemia occurs in the first day of life until the normal intestinal bacterial flora become established.

Combined prothrombin and stable factor deficiency is also induced by di coumarin and related drugs the predominating defect being in the stable factor

The clinical response in two children with PTC deficiency treated with small transfusions of serum (100 to 200 ml of serum previously frozen and stored at -10°C) was more prompt and prolonged than with whole blood or fresh frozen plasma^{10a}

Plasma Thromboplastin Antecedent Deficiency (Hemophilia C)

Plasma thromboplastin antecedent deficiency is due to a deficiency of PTA which is required for thromboplastin formation and occurs as an inherited abnormality^{99, 100} In contrast to classic hemophilia and PTC deficiency this form of hemophilia is transmitted as a dominant with no sex linkage Sporadic cases are observed as is true of the other hemophilias described

Clinical and Laboratory Features The hemorrhagic tendency is usually milder than in patients with AHG or PTC deficiencies Spontaneous hemorrhage is rare but may occur as in a recently reported patient with cerebral hemorrhage⁶ Usually bleeding is related to trauma Because of the infrequency of spontaneous bleeding and the relative mildness of the bleeding PTA deficiency often escapes detection As in patients with the other types of hemophilia excessive bleeding may follow minor injuries and surgical procedures usually dental extraction and tonsillectomy Easy bruising and occasionally spontaneous epistaxis have been noted but hemarthroses are uncommon

Usually the whole blood clotting time is normal or only slightly prolonged and except in the most mildly affected patients prothrombin consumption is abnormal The deficiency of PTA is detected by the thromboplastin generation test (abnormality in both the serum and plasma component as contrasted with the plasma component alone in AHG deficiency and serum component alone in PTC deficiency)

Treatment Plasma thromboplastin antecedent is very stable when stored permitting the use of banked blood or plasma The amounts to be given are similar to those used in the treatment of AHG and PTC deficiency with intervals of twenty four to forty eight hours This applies to treatment of the acute bleeding emergency as well as to preparation for a surgical procedure Here too fresh frozen plasma is often given because of its greater availability

DISORDERS DUE TO A DEFICIENCY OF FACTORS REQUIRED FOR THE CONVERSION OF PROTHROMBIN TO THROMBIN (PHASE 2 OF COAGULATION)

General Considerations Disturbances in the second stage of coagulation result in a deficient formation of thrombin Both intrinsic (plasma) and extrinsic thromboplastin (prothrombinase) activate the conversion of prothrombin to thrombin in the presence of calcium so that both mechanisms play an important part in hemostasis It has already been stated that the formation of extrinsic thromboplastin depends upon the interaction of tissue extract and labile (V) stable (VII) and Stuart Prower factors The deficiency of these factors as well as of prothrombin is most conveniently determined in the laboratory in connection with the second stage of coagulation The prothrombin complex there

and in the presence of liver disease leukemia malignancies pernicious anemia and abruptio placentae Factor V deficiency has been reported in patients with hemorrhagic scarlet fever (purpura fulminans) ⁶⁰

Stable Factor Deficiency (Factor VII Deficiency, Proconvertin Deficiency Congenital Hypoproconvertinemia)

Isolated stable factor deficiency has been reported ^{10 40 64 65} but its incidence is rare. Affected patients give a history of excessive bleeding dating from early life ¹³. Hemorrhagic phenomena are seen in patients with factor VII concentrations of approximately 10 per cent of normal or less ². Bruising, marked epistaxis, hematuria, hemarthrosis, melena, hematoma, and often bleeding after dental extraction are common phenomena in this group. Spontaneous hemorrhages have been described in the central nervous system especially subarachnoid bleeding. The disease is familial and is transmitted as a simple dominant. A decreased activity of stable factor may be found in relatives who show greatly decreased amounts of this factor in the blood. In one family, intracranial hemorrhage was the cause of death of the father and the siblings ⁶.

The one stage plasma prothrombin time of Quick is prolonged and is corrected by the addition of serum (fresh or stored) which contains stable factor but is not corrected by prothrombin free plasma (barium sulfate treated). The bleeding and whole blood clotting time may be normal or prolonged. Clotting time is normal in the presence of small amounts of stable factor but is prolonged in its complete absence. Acquired stable factor deficiency occurs in patients with many pathologic conditions probably always with some prothrombin defect (such as are caused by dicoumarin and tromexan therapy).

Treatment consists of the transfusion of banked blood or plasma. Multiple transfusions are usually necessary. Serum which is a rich source of factor VII is very effective. The amounts to be given can be controlled by the reduction in prothrombin time. Vitamin K is ineffective since it will not correct a congenital defect in which the inability of the end organ, the liver, to synthesize prothrombin and stable factor exists.

Hemorrhagic Disease of the Newborn Infant (Hypoprothrombinemia in the Newborn Infant)

Hemorrhagic disease of the newborn infant is a self limited hemorrhagic disorder of the first days of life caused by a marked deficiency of prothrombin ⁷ and stable factor ⁸. There is strong evidence that PTC and Stuart Prower factors which are associated with plasma thromboplastin formation are also deficient in hemorrhagic disease of the newborn infant ^{31 6}.

Etiology—Prothrombin and Stable Factor Deficiency. Hemorrhagic disease of the newborn infant represents an exaggeration of prothrombin and stable factor deficiencies events which normally occur in the first days of life. Prothrombin and stable factor values already diminished at birth fall sharply in the second to the fourth days returning to normal spontaneously at seven to ten days.

Stable factor is more markedly reduced than prothrombin and accounts pri-

These agents act as anticoagulants by interfering with the hepatic synthesis of prothrombin and similar factors. During the first days of treatment with any of the dicoumarin group of drugs it is probable that stable factor deficiency is the main cause of the lengthened one stage prothrombin time.¹

Congenital Deficiencies Congenital deficiencies represent coagulation disorders resulting from isolated deficiencies of one of the factors of the prothrombin complex. The bleeding manifestations are similar to those observed in patients with disorders in which multiple deficiencies exist and will be described later. In the congenital deficiencies (prothrombin and labile, stable and Stuart Prower factors) vitamin K therapy is uniformly unsuccessful.

Idiopathic (Congenital) Hypoprothrombinemia Congenital prothrombin deficiencies are rare but authentic cases of failure to synthesize this protein have been reported. There may be a family history of idiopathic hypoprothrombinemia with hemorrhages appearing in childhood and bleeding from mucous surfaces and into skin and tissues. Since the majority of patients with hypoprothrombinemia usually possess associated deficiencies it is important to rule out labile and stable factor involvement especially the latter²⁴ before the diagnosis of true hypoprothrombinemia is justified.

Labile Factor Deficiency (Factor V Deficiency, Parahemophilia, Owren's Disease)

Originally described by Owren^{56, 57} and since by others,⁹ labile factor deficiency is detected by a prolonged one stage prothrombin time with undiluted plasma which is corrected when the patient's plasma is mixed with deprothrombinized (barium sulfate-absorbed) normal human plasma. The coagulation time of whole blood is prolonged and the bleeding time may be prolonged or normal. In patients with labile or stable factor deficiencies bleeding is usually from the mucous membranes and skin. In both conditions the bleeding tendency dates from infancy but may not be manifested until later childhood after trauma or a surgical procedure such as tonsillectomy. Many newborn infants have low levels of labile factor during the first two days of life which rise toward normal by the sixth day.⁶³ A syndrome of congenital labile factor deficiency with syndactylism has been reported. In this family with normal parents five of seven siblings had syndactylism and a bleeding tendency. In three of the children a deficiency of the labile factor in the plasma was demonstrated.⁷⁰

Labile factor deficiency is a familial disease affecting both sexes and is transmitted as an incompletely dominant gene.⁴ It has also been suggested⁶⁹ that the plasma of the homozygous patient is almost entirely lacking in labile factor and the patient is a bleeder. However the heterozygous person has only a partial deficiency and is usually not a bleeder. The disease may be of variable intensity. The only treatment of known value is transfusion with fresh normal blood or plasma because of the lability of the factor. The abnormality is not affected by vitamin K. Acquired labile factor deficiency occurs in persons with a variety of clinical conditions usually in association with prothrombin deficiency since both factors are affected simultaneously after surgical operations.

and in the presence of liver disease leukemia malignancies pernicious anemia and abruptio placentae Factor V deficiency has been reported in patients with hemorrhagic scarlet fever (purpura fulminans) ⁶⁹

Stable Factor Deficiency (Factor VII Deficiency, Proconvertin Deficiency, Congenital Hypoproconvertinemia)

Isolated stable factor deficiency has been reported ^{1, 4, 55, 51b} but its incidence is rare. Affected patients give a history of excessive bleeding dating from early life ¹³. Hemorrhagic phenomena are seen in patients with factor VII concentrations of approximately 10 per cent of normal or less. Bruising, marked epistaxis, hematuria, hemarthrosis, melena, hematoma, and often bleeding after dental extraction are common phenomena in this group. Spontaneous hemorrhages have been described in the central nervous system, especially subarachnoid bleeding. The disease is familial and is transmitted as a simple dominant. A decreased activity of stable factor may be found in relatives who show greatly decreased amounts of this factor in the blood. In one family intracranial hemorrhage was the cause of death of the father and the siblings ⁴.

The one stage plasma prothrombin time of Quick is prolonged and is corrected by the addition of serum (fresh or stored) which contains stable factor but is not corrected by prothrombin free plasma (barium sulfate treated). The bleeding and whole blood clotting time may be normal or prolonged. Clotting time is normal in the presence of small amounts of stable factor but is prolonged in its complete absence. Acquired stable factor deficiency occurs in patients with many pathologic conditions, probably always with some prothrombin defect (such as are caused by dicoumarin and tromexan therapy).

Treatment consists of the transfusion of banked blood or plasma. Multiple transfusions are usually necessary. Serum which is a rich source of factor VII is very effective. The amounts to be given can be controlled by the reduction in prothrombin time. Vitamin K is ineffective since it will not correct a congenital defect in which the inability of the end organ, the liver, to synthesize prothrombin and stable factor exists.

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marily for the prolonged one stage prothrombin time⁴³ The deficiency of these coagulation factors especially stable factor is more pronounced in the premature than in the full term infant and the return to normal takes place more slowly

Several factors contribute to the genesis of physiologic prothrombin and stable factor deficiencies the bacteria free nature of the newborn infant's intestinal tract which prevents vitamin K synthesis until intestinal flora are established with food ingestion liver immaturity ■ lack of a reserve supply of the vitamin and in some cases ■ depletion of vitamin K in the mother due to poor dietary intake poor absorption and impaired utilization due to liver dysfunction

Concentrations of 20 to 25 per cent of prothrombin and stable factor are critical values below which a potential hemorrhagic state exists Values of 5 per cent and lower occur in infants with hemorrhagic disease In the majority of patients bleeding occurs spontaneously during the period when the prothrombin and stable factor concentrations are normally reduced The incidence of hemorrhagic disease of the newborn infant is given variously as about 1 in 200 to 400 births but it appears much less commonly at present perhaps due to the widespread use of vitamin K

A deficiency of PTC (active in the first phase of coagulation) occurs in combination with prothrombin and stable factor deficiencies in the newborn period^{44,45} PTC deficiency follows the pattern of the prothrombin complex in the first weeks of life with its most marked decrease about the third day of life and spontaneously increasing thereafter Aballi and co workers observed marked improvement in PTC concentration by administering vitamin K to full term infants but an inadequate response in the premature infant Because of the slowness with which prothrombin stable factor and PTC return to non hemorrhagic levels in the premature infant and because of the inadequacy of vitamin K therapy it may be safer to defer circumcision for a period of three to four weeks

Clinical and Laboratory Features Bleeding is rarely massive consisting of persistent oozing from the umbilical cord and mucous surfaces hematemesis hematuria gastrointestinal and vaginal bleeding petechiae ecchymoses and infrequently spontaneous central nervous system and meningeal hemorrhage Serious hemorrhage can occur from minor trauma Circumcision may result in slow and continued bleeding³⁰ There is suggestive evidence that the PTC deficiency is especially responsible for the prolonged coagulation time and the tendency to bleed encountered in patients with this disease

The platelet count is normal and anemia ■ proportional to blood loss In addition to prolonged prothrombin time prolonged coagulation and bleeding time occasionally occur but are not necessary for the diagnosis The presence of an associated vascular lesion is suggested by the prolonged bleeding time rarely positive tourniquet test and evidence of skin hemorrhages

Differential Diagnosis The stools of many infants are benzidine positive indicating the presence of blood A markedly prolonged prothrombin time differentiates hemorrhagic disease of the newborn infant from blood derived from maternal sources such as blood swallowed at birth or while nursing from fissured nipples In a grossly bloody stool this test is helpful but what is even more specific is the identification of the source of blood as to its maternal or fetal nature

The application of the alkali denaturation test distinguishes between maternal blood (adult type) and infant's blood (fetal type) the latter being more resistant to denaturation with alkali than adult hemoglobin.⁴ Anticoagulant therapy in the mother may provoke serious bleeding in the fetus and newborn infant. Conditions other than the hypoprothrombinemias deserve consideration in the differential diagnosis. Disorders of thromboplastin formation and afibrinogenemia can be eliminated by appropriate tests. Oozing from the umbilical cord occurs in infants with hemorrhagic disease but is unusual in those with hemophilia. Infants with congenital thrombocytopenic purpura, congenital syphilis, bacteremia and hemorrhagic manifestations of erythroblastosis fetalis also present clinical bleeding in the first days of life.

Treatment. The discovery of vitamin K by Dam⁵ and of its relationship to prothrombin formation led to its use in pregnant women before delivery and in the infant at birth to prevent hypoprothrombinemia. In recent years, however, doubt has been thrown on the value of vitamin K in the prevention⁶ and management⁷ of hemorrhagic disease of the newborn infant. In the premature infant especially the shortage of vitamin K is not so important as the inadequate synthesis of the clotting factors (prothrombin and stable factor) caused by immaturity of the liver. Occasionally, prothrombin levels have been raised by vitamin K given to the mother or infant but this did not reduce the incidence of the disease. There is ample evidence, however, that the administration of vitamin K to the newborn infant arrests the fall of prothrombin and stable factor although normal levels may not be achieved.¹⁰ In another series, vitamin K administration corrected all the clotting defects (stable factor, PTC and Stuart Prower factor deficiencies and hypoprothrombinemia) associated with hemorrhagic disease of the newborn infant.⁸ Until a more effective means of controlling hemorrhagic disease of the newborn infant with its potentially serious clinical manifestations becomes available, vitamin K therapy still remains the agent of choice for prophylaxis and treatment.

Vitamin K. The large number of vitamin K preparations may be classified as those containing oil soluble vitamin K and the synthetic analogues, many of which are water soluble. One of the very effective natural vitamin K products, K₁, phytonadione (Konakion® Mephyton¹) can be given intramuscularly, intravenously or as a tablet for oral use. Two synthetic water soluble vitamin K preparations which are in common use are menadiol sodium bisulfate (Hyknone²) and menadiol sodium diphosphate (Synkavite³). They are available for oral use and subcutaneous, intramuscular and intravenous injection.

Excessive doses of vitamin K are to be avoided because of their known hemolytic action and tendency to cause hyperbilirubinemia especially in the premature infant. It has therefore been recommended by the Council on Drugs of the American Medical Association¹¹ that as an equivalent of 1 mg. of synthetic vitamin K₁, menadiol is adequate to prevent hemorrhagic disease of the newborn infant.

Roch Laboratories, Nutley, N. J.
 McKesson Sharp & Dohme Inc., Philadelphia, Pa.
 Abbott Laboratories, North Chicago, Ill.

The natural K_1 vitamins act more rapidly than the synthetic preparations and raise plasma prothrombin to therapeutic levels within two to four hours. Phytonadione is of special value in threatening or actual hemorrhage. Prophylaxis of hemorrhagic disease of the newborn infant is achieved by the use of phytonadione (Mephyton) or other natural or synthetic vitamin K (menadione) by giving the mother daily doses of 2 to 4 mg orally for one week before delivery or parenteral injection of an equivalent dose of one of the water soluble preparations several hours before delivery. For the infant at birth phytonadione is effective when given intramuscularly in a 1 to 2 mg dose. The water soluble forms of vitamin K (Hykinone Synkavite) are however completely adequate in the presence of vitamin K deficiency states.

In our own institution normal newborn infants are not given vitamin K routinely. A dose of 1 to 2 mg of vitamin K_1 (phytonadione) is given intramuscularly to all premature infants and infants in all weight groups exhibiting marked fetal distress and anoxia. Routine administration of vitamin K to antepartum mothers has been discontinued. We have not been impressed with any increased incidence of hemorrhagic disease of the newborn infants since discontinuance of vitamin K administration as a routine measure in parturient mothers of full term infants.

The danger of hyperbilirubinemia in the infant from excessive dosage of vitamin K given to the mother has been noted. The parenteral administration of 72 mg of a vitamin K analogue (menadione sodium bisulfate Hykinone) during labor resulted in bilirubin levels in the newborn premature infant ranging from 21 to 47 mg per 100 ml with neurologic sequelae in two patients.

Transfusions of blood or plasma are given to replenish prothrombin and stable Stuart Prower and PTC factors and in severely affected patients to combat anemia and shock. Fresh human serum (in 5 ml amounts) is especially recommended as a therapeutic adjuvant in the severely affected patient because of its readily available content of stable factor which may be especially involved. Although external bleeding can be controlled, internal bleeding results in an appreciable mortality (estimated at 70 per cent).

Stuart Prower Factor Deficiency

The Stuart Prower factor^{36, 106} functions both in the formation of intrinsic and extrinsic thromboplastin. Deficiencies of the factor have been found in patients with liver disease, in newborn infants with hemorrhagic disease, and in patients receiving coumarin compounds.³ The deficiency is transmitted as an incompletely recessive autosomal character. In the heterozygote this factor is reduced on an average to 36 per cent and causes mild bleeding. The homozygote has a moderately severe hemorrhagic disorder manifested by epistaxis, hematomas, and hemarthrosis. A deficiency of Stuart Prower factor is an important part of the clotting defect of premature¹⁰⁶ and full term infants.³

Multiple Defects

Capillary and Single Coagulation Factor Deficiency. Vascular hemophilia (pseudohemophilia B) represents a dual hemostatic disorder in which a capillary defect responsible for clinical bleeding is combined with an AHC deficiency.

The vascular defect alone is responsible for an increased bleeding time and often a positive tourniquet test. Capillary disorders have also been found to coexist with either labile or stable factor deficiencies (factors V and VII respectively) with congenital afibrinogenemia and with double factor deficiencies.¹¹⁴

Multiple Factor Deficiencies A combined deficiency of AHG and PTC⁹ and of congenital labile factor with AHG deficiency has also been described.⁶¹

DISORDERS DUE TO A DEFICIENCY OF FIBRINOGEN (PHASE 3 OF COAGULATION)

A deficiency of fibrinogen is a rare coagulation defect resulting in a profound disturbance of the blood clotting mechanism. The complete deficiency is congenital whereas conditions with reduced amounts of fibrinogen (hypofibrinogenemia or fibrinogenopenia) are either congenital or acquired.

Congenital Afibrinogenemia

Congenital afibrinogenemia is marked by a complete or practically complete and permanent absence of fibrinogen from the blood resulting in a hemorrhagic syndrome resembling hemophilia except that it affects either sex.⁹ The patient is frequently the offspring of a consanguineous marriage. An incomplete recessive or semidominant inheritance⁶² is postulated since low fibrinogen levels are occasionally found in the parents of affected children.⁴⁹

Bleeding is manifest from birth with excessive oozing from the umbilical cord or from the navel on separation of the cord and after circumcision. Persistent bleeding follows trauma, surgical procedures and the loss of deciduous teeth. Excessive bruising and ecchymosis are common. In contrast to hemophilia hemarthroses are rare.⁹ Joint disability does not result even in this circumstance probably due to a lack of fibrin formation. Except for the absence of fibrinogen the other phases of clotting are normal. Despite incoagulable blood long intervals with freedom from hemorrhage are common¹¹ and the condition is usually associated with only a minor degree of disability.

Congenital Hypofibrinogenemia

Reduced fibrinogen levels may be present in parents of children with afibrinogenemia in whom bleeding is absent or in patients with "constitutional fibrinogenemia" in whom there is a tendency toward abnormal bleeding.

Acquired Fibrinogen Deficiency

Acquired fibrinogen deficiencies have been noted in patients with many conditions such as severe liver disease, bone marrow involvement with invasion by leukemic or tumor cells, polycythemia vera, malignancies, tuberculosis, shock, burns, transfusion reactions,¹¹ and obstetric complications.

Hypofibrinogenemia in Patients With Congenital Heart Disease

A potential hemorrhagic diathesis in congenital cyanotic heart disease has been described.¹¹ Marked impairment of clot retraction is due to a combination of thrombocytopenia and low blood fibrinogen in the presence of a high hemato-

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time. Hence if the whole blood clotting time is prolonged and the Quick one stage prothrombin time shows no clotting or a very incomplete clot total or partial fibrinogen should be suspected.¹ It has been estimated that if the prothrombin time of a given plasma sample is normal its fibrinogen level must be in excess of 100 mg per cent.¹¹

In hypofibrinogenemia the clotting time may be normal or slightly prolonged depending upon the content of fibrinogen. With reduced fibrinogen levels clotting occurs promptly and retraction of the clot results in a relatively large volume of erythrocytes over which is suspended a very small mass. Eventually the clot becomes detached and drops into the erythrocyte suspension by which it is obscured.

Treatment of the Fibrinogen Deficiencies

The normal concentration of fibrinogen in the blood is 250 to 400 mg per 100 ml. Only in the event of a hemorrhagic episode is treatment necessary and this consists of the administration of blood plasma and concentrated fibrinogen (Cohn fraction 1). Approximately 0.2 to 0.3 gm of fibrinogen is present in 100 ml of plasma and about 0.7 to 0.9 gm in 1 pint of whole blood. Concentrated human fibrinogen preparations are now in good supply and constitute the first choice of treatment. In contrast to the labile antihemophilic globulin fibrinogen remains stable when stored. In critical situations in which severe and persistent bleeding is associated with marked fibrinogen depletion substantial amounts of human fibrinogen may be given (up to 4 gm intravenously in children). It must be cautioned however that the use of fibrinogen carries a definite hazard of hepatitis.

A critical blood level of 80 mg of fibrinogen per 100 ml is required for effective hemostasis and treatment should be directed toward achieving at least this concentration. Gitlin and Borges⁶ determined immunochemically that one half of administered fibrinogen disappeared from the circulation in the first forty eight hours. After the first two days the fibrinogen follows a logarithmic decay curve with a half life of four days indicating that the fundamental defect in these patients is a failure to synthesize adequate amounts of fibrinogen. Fibrinogen labeled with radioactive iodine ¹²⁵I shows a mean half life of 5.1 days (range 4.1 to 6 days).³ A dose of 100 mg of fibrinogen per kilogram of body weight raises the fibrinogen concentration of a patient with afibrinogenemia to approximately 200 mg per 100 ml.¹¹ With the use of plasma the fibrinogen component may remain in the circulation for as long as four days and with concentrated fibrinogen it may remain twice as long.⁴

In patients with acute fibrinolysis treatment is urgent and consists of prompt administration of whole blood plasma and concentrated fibrinogen (fraction 1). Use of ACTH and the steroid hormones has been suggested as a means of increasing antifibrinolytic activity^{10a} and controlling bleeding.

CIRCULATING ANTICOAGULANTS

Naturally Occurring Anticoagulants The fluidity of the circulating blood depends upon the integrity of vascular endothelium and the presence of the

crit The slight to moderate prolongation of prothrombin time is not a cause of the hemorrhagic disorder Although these abnormal findings were present in patients in all age groups postoperative hemorrhage was chiefly a problem in adults and adolescents Children however with evidence of marked impairment revealed by hematologic tests underwent operation without hemorrhage In adults with polycythemia vera as well as in patients with secondary polycythemia of congenital heart disease the hemorrhagic tendency is attributed in large measure to fibrinogenopenia The treatment preoperatively in emergency situations in patients with polycythemia is liberal venesection together with the return of normal plasma to the patient ¹⁰⁹

Incoagulability of the blood as a result of hypofibrinogenemia is a well known complication of several obstetric conditions: amniotic fluid infusion (embolism) premature separation of the placenta and prolonged retention of a dead fetus One of the explanations for the deficiency in fibrinogen is that a thromboplastin like material associated with these complications gains access to the maternal circulation resulting in fibrinogen consumption Another theory is that as fibrin is formed intravascularly it is destroyed by a fibrinolysin released as a result of shock ¹¹

Hypofibrinogenemia has been encountered among the sensitized mothers of severely ill and stillborn infants in the course of erythroblastosis It has been pointed out that the threat of developing afibrinogenemia in these patients may necessitate early delivery ¹²

Fibrinolysis (Fibrinolytic Purpura)

The fibrinolytic process is one in which the living organism dissolves blood clots and disposes of fibrin ¹³ This is effected through a complex enzymatic system The activation of enzymes capable of destroying fibrin occurs acutely in patients with trauma hemorrhagic shock extensive burns transfusion reactions and obstetric accidents and chronically in those with leukemia liver disease and disseminated carcinoma

Fibrinolysis accounts for recanalization of a blood vessel after hemostasis has been completed Disruption of the hemostatic mechanism in patients with carcinoma or after extensive surgery with severe bleeding manifestations (fibrinolytic purpura) results from enzymatic digestion of fibrin clot fibrinogen and other proteins involved in blood coagulation ¹⁰⁸ In patients with hemorrhage following abruptio placentae and after surgery for cirrhosis of the liver the primary causative factor of afibrinogenemia is fibrinolysis ⁹⁰

Fibrinolysis depends upon the liberation into circulation of tissue kinases which activate preformed and inert precursors (profibrinolysins) In another terminology the precursor plasminogen is converted to the active proteolytic enzyme (plasmin) by various activation systems (fibrinokinase or tissue activators derived from tissues) ¹⁴ The mechanism of activation is obscure Excessive fibrinolysis frequently results in severe bleeding from multiple areas

Laboratory Findings in Fibrinogen Deficiencies

In complete afibrinogenemia the whole blood is incoagulable The absence of fibrinogen is confirmed by the failure of citrated blood or plasma to clot when thrombin is added and by the absence of a precipitate when the plasma is heated to 56° C ¹ If the plasma remains clear afibrinogenemia exists since fibrinogen precipitates at about this temperature

The blood of patients with hemophilia shows a normal one stage prothrombin

may be necessary however in the face of continued bleeding although there is the risk of generating increased amounts of anticoagulant ACTH and steroids have been of limited value. However the anticoagulant has been known to disappear spontaneously or the bleeding may be minimal despite its presence.

SUMMARY OF REPLACEMENT THERAPY OF THE COAGULATION DISORDERS

The frequency of plasma administration depends upon the life span of the deficient factor in the patient's circulation, loss of the factors through utilization, the increased requirements with active bleeding, consumption by internal hemorrhage or possibly by neutralization or destructive action of anticoagulants.⁹⁸ When the critical level of a particular factor in a patient with any bleeding disorder (of the first or second stage of coagulation) approximates that observed in those with hemophilia due to AHG deficiency, comparable amounts of plasma are given initially (10 ml per kilogram of body weight). The intervals between the administration however may be increased depending upon the survival of a particular factor. Thus except for AHG the intervals of administration may be lengthened to twenty-four to forty-eight hours or longer for patients with PTC or PTA deficiency and for those with the hypoprothrombinemias.

Fibrinogen may be administered in the form of plasma. Since 100 ml of plasma supplies 10 mg of fibrinogen, this dosage must be repeated to bring the total to 80 mg per cent or over as required for hemostasis. The slow destruction in the blood stream reduces the need for frequent plasma administration.

For surgery in patients with one of the hemophilias, plasma is given thirty minutes before the operation to be repeated every four to six hours in patients with AHG deficiency and every twelve to twenty-four hours in those with PTC or PTA deficiency. After the first twenty-four hours plasma is given to patients with AHG deficiency in accordance with the specifications of the schedule given previously in this chapter and every forty-eight hours to seventy-two hours to those with PTC or PTA deficiency. The type of plasma to be used, whether fresh or stored, is indicated in the following list:

1. Fresh whole blood or plasma
 - A. Thrombocytopenic purpura (whole blood or platelet rich plasma collected in silicized or plastic equipment)
 - B. Hemophilia (hemophilia A, AHG deficiency)
 - C. Parahemophilia (labile or factor V deficiency)
 - D. von Willebrand's disease
 - (1) Vascular defect only (pseudohemophilia A)
 - (2) Vascular defect with AHG deficiency (vascular hemophilia, pseudohemophilia B)
2. Fresh or stored whole blood or plasma
 - A. Fibrinogen deficiency
 - B. PTC deficiency (hemophilia B, Christmas factor)
 - C. PTA deficiency
 - D. Factor VII deficiency (stable factor)
 - E. Stuart-Prower factor deficiency

natural anticoagulants plasma antithrombin heparin and a postulated antithromboplastin

Antithromboplastin has been described as a lipid inhibitor an excess of which has been held accountable for hemophilia²¹ It has been shown for instance that contact of hemophilic plasma with asbestos or glass will normalize its clotting by adsorption of the inhibitor On the other hand transfusion of hemophilic plasma treated by removal of the inhibitor in this manner or by extraction with ether still demonstrates a deficiency of AHG in *in vivo* experiments Nevertheless the presence of an inhibitor in hemophilic plasma cannot be discounted Proof of the presence of circulating anticoagulant is an intrinsic feature of the hemophilic defect within the circulation awaits further corroborative evidences⁷

Acquired Anticoagulants The majority of acquired inhibitors prevent the formation of thromboplastin either as a complication of hemophilia or causing a hemorrhagic diathesis resembling hemophilia²² The best defined blood clotting inhibitors are those which appear in the blood of the patient with hemophilia following repeated transfusions This has been ascribed to an immunologic response to the antihemophilic globulin in blood or plasma Similar inhibitors of plasma thromboplastin component (PTC) have also been described⁴

Acquired hemophilia like disease based on circulating anticoagulants has also been recorded after pregnancy In one case the anticoagulant was detected in the patient's child during the first two and one half months of life indicating its transplacental transfer⁴⁸ Circulating anticoagulants have appeared in previously healthy male and female patients or in connection with an associated illness notably lupus erythematosus periarthritis nodosa rheumatoid arthritis tuberculosis and glomerulonephritis Hemorrhagic disease due to a rise in blood heparin or substances with heparin like activity has appeared after treatment with physical or chemical reagents such as nitrogen mustard and after exposure to ionizing radiation and probably explains the decreased coagulability of the blood in patients with anaphylaxis

The presence of a circulating anticoagulant contributes to the diagnosis of lupus erythematosus This abnormality may disappear fairly rapidly during steroid therapy⁴⁹ In one patient with this disease severe bleeding was associated with hypoprothrombinemia plus an anticoagulant active against formed blood and tissue prothrombinase⁵⁰

Tests for Anticoagulants A screening test for the presence of clotting inhibitors is an integral part of a complete coagulation study Patients with circulating anticoagulant usually show prolonged clotting and recalcification time Although normal whole blood or plasma will correct a deficiency the presence of an anticoagulant in the patient's blood or plasma will prolong the normal clotting time The screening test consists of mixing various amounts of the patient's blood or plasma with normal blood or plasma and determining the clotting and recalcification times of the mixtures¹⁰⁴ Circulating anticoagulants can also be detected by the thromboplastin generation test by mixing either the patient's serum or adsorbed plasma with the corresponding normal reagent With an inhibitor little or no thromboplastin is generated³

Treatment Attempts to overcome the refractory state by repeated transfusions with massive amounts of blood have been of questionable success This measure

events in pediatric practice. Prothrombin consumption and thromboplastin generation tests provide accurate information concerning basic defects in coagulation but are obviously not suited for routine use. The most practical course is to obtain a detailed personal and family history of hemorrhage.

As a general rule a hemorrhagic disorder is suspected when the degree of bleeding is out of proportion to the extent of trauma. This circumstance usually becomes apparent by the time the child is walking without protection. More complete studies are warranted in the patient with a history of severe and uncontrollable epistaxis, easy bruising, hematomas especially in protected areas of the body, and excessive bleeding from cuts, skin prick as for blood counts and injections, and during the eruption or loss of deciduous teeth. Importance is attached to a history of prolonged bleeding or transfusions after an operative procedure in a sibling or parent. The absence of bleeding after a dental extraction of moderate severity or after tonsillectomy usually indicates the absence of congenital disease.

The clotting time is only of value in the older child in whom a vein can be entered without difficulty. Capillary tests are unreliable because of the amount of tissue juice containing thromboplastin which enters the peripheral blood sample. Since a normal clotting time will be found in patients mildly affected with hemophilia and often in those with conditions with defects in prothrombin conversion, the history becomes increasingly important. Short of the use of more complicated tests, the bleeding history is the most useful means of anticipating difficulty in patients with sporadic AHG and PTC deficiency in whom there is no family history (25 to 30 per cent of patients with hemophilia).

In addition to the history, a complete physical examination and examination of the blood smear for platelets to rule out thrombopenic states at the time of routine preoperative hemoglobin determinations and white cell counts constitute effective screening devices. For these reasons, a careful history is essential and it has even been suggested that because of inherently misleading results²⁹ routine presurgery tests of bleeding and clotting times be abandoned.

LABORATORY INVESTIGATION OF COAGULATION DISORDERS

General Considerations. Investigation for a hemorrhagic disorder is usually prompted by the clinical history and physical findings which also give direction to the scope of the laboratory work-up. The tests consist of the routine coagulation procedures for screening and more elaborate devices for the detection of the specific causative defect.

In evaluating the results of routine coagulation tests which may be the only ones available in a laboratory, it should be remembered that patients with the mild bleeding disorders may show few or no abnormalities upon routine testing. In such instances a careful history will compel attention toward the need for more intensive investigation.

In the history of the patient or other members of his family, those episodes are important in which bleeding from minor cuts and scratches and after dental extraction and tonsillectomy is prolonged with or without the need for trans-

Data relative to plasma requirements in deficiencies of coagulation factors are as follows

- 1 Stable when stored
 - A PTC
 - B PTA
 - C Prothrombin
 - D Stable factor (factor VII)
 - E Fibrinogen
- 2 Labile when stored
 - A AHG
 - B Labile factor (factor V)
- 3 Survival time of clotting factors in circulation of patient²⁴
 - A AHG—12 to 24 hours
 - B PTC—48 to 72 hours
 - C PTA—24 hours
 - D Prothrombin—36 to 42 hours
 - E Stable factor—24 to 36 hours
 - F Labile factor—24 to 48 hours
 - G Fibrinogen—4 days

EPISTAXIS

Recurrent nosebleeds constitute a common problem in pediatric practice. They usually occur spontaneously, often at night and frequently in the course of an upper respiratory infection. They are usually controlled by simple measures such as digital pressure or cotton packs rimmed with petroleum jelly or soaked in Adrenalin, but sometimes they require cauterization and nasal packing. Bleeding, however, may be profuse and blood loss may be substantial.

The localization and causation of nosebleeds assume importance when tonsillectomy and adenoidectomy are contemplated. As an isolated symptom, it is rare in patients with hemophilia. In those with intractable nosebleeds, pseudo-hemophilia should be considered. The platelet count is usually normal in patients with idiopathic epistaxis and depressed in those in whom epistaxis accompanies leukemia, aplastic anemia, or conditions associated with hypersplenism such as Gaucher's disease. Nosebleeds infrequently constitute the sole clinical feature of either idiopathic thrombocytopenic purpura or classic (AHG deficient) hemophilia. Epistaxis may also occur in patients during the course of measles, rheumatic fever, systemic lupus erythematosus, typhoid fever, glomerulonephritis, sickle cell anemia, and Cooley's anemia.

From present evidence it appears safe for patients with epistaxis without a history of abnormal bleeding to be operated upon without further tests. This means that in neither the patient nor any member of his family is there a history of easy bruising or persistent bleeding after dental extraction, circumcision, immunization, or eruption or loss of deciduous teeth. Furthermore, physical examination should reveal no petechiae, purpura, or ecchymoses.

PROCEDURE FOR SCREENING POTENTIAL BLEEDERS

Adequate screening devices before surgery are especially important in infants and children since circumcision, dental extraction, and tonsillectomy are common

events in pediatric practice. Prothrombin consumption and thromboplastin generation tests provide accurate information concerning basic defects in coagulation but are obviously not suited for routine use. The most practical course is to obtain a detailed personal and family history of hemorrhage.

As a general rule a hemorrhagic disorder is suspected when the degree of bleeding is out of proportion to the extent of trauma. This circumstance usually becomes apparent by the time the child is walking without protection. More complete studies are warranted in the patient with a history of severe and uncontrollable epistaxis, easy bruising, hematomas, especially in protected areas of the body, and excessive bleeding from cuts, skin prick as for blood counts and injections, and during the eruption or loss of deciduous teeth. Importance is attached to a history of prolonged bleeding or transfusions after an operative procedure in a sibling or parent. The absence of bleeding after a dental extraction of moderate severity or after tonsillectomy usually indicates the absence of congenital disease.

The clotting time is only of value in the older child in whom a vein can be entered without difficulty. Capillary tests are unreliable because of the amount of tissue juice containing thromboplastin which enters the peripheral blood sample. Since a normal clotting time will be found in patients mildly affected with hemophilias and often in those with conditions with defects in prothrombin conversion the history becomes increasingly important. Short of the use of more complicated tests the bleeding history is the most useful means of anticipating difficulty in patients with sporadic AHG and PTC deficiency in whom there is no family history (25 to 30 per cent of patients with hemophilia).

In addition to the history, a complete physical examination and examination of the blood smear for platelets to rule out thrombopenic states at the time of routine preoperative hemoglobin determinations and white cell counts constitute effective screening devices. For these reasons a careful history is essential and it has even been suggested that because of inherently misleading results⁸⁷ routine presurgery tests of bleeding and clotting times be abandoned.

LABORATORY INVESTIGATION OF COAGULATION DISORDERS

General Considerations. Investigation for a hemorrhagic disorder is usually prompted by the clinical history and physical findings which also give direction to the scope of the laboratory work up. The tests consist of the routine coagulation procedures for screening and more elaborate devices for the detection of the specific causative defect.

In evaluating the results of routine coagulation tests which may be the only ones available in a laboratory, it should be remembered that patients with the mild bleeding disorders may show few or no abnormalities upon routine testing. In such instances a careful history will compel attention toward the need for more intensive investigation.

In the history of the patient or other members of his family those episodes are important in which bleeding from minor cuts and scratches and after dental extraction and tonsillectomy is prolonged with or without the need for trans-

fusions. In the infant more than normal oozing of blood after circumcision and from cuts of the tongue and lips protruded oozing of blood from the gums during the eruption of deciduous teeth and bleeding at the site of immunization are noteworthy. The presence of extensive bruising observed during physical examination is a sign of recurrent hemorrhage. The bruising may represent a disorder in which platelets are deficient.

Both hematologic and nonhematologic disturbances may give evidence of gross bleeding and must be differentiated from a basic bleeding tendency. Epistaxis may be a feature of thrombocytopenic purpura and leukemia and hematemesis and melena may be features of Bant's disease. Severe epistaxis a frequent occurrence in childhood deserves a complete coagulation study if it is associated with easy bruising and a suspicious bleeding history. On the other hand epistaxis per se without other evidence of bleeding or a negative family or personal history of bleeding has in our experience not justified more than the routine laboratory examinations. For all practical purposes a patient who has undergone tonsillectomy or tooth extraction without evidence of abnormal bleeding may be considered not to have hemophilia or any other serious first phase coagulation defect.

Screening Tests The screening tests for a suspected hemorrhagic disorder include coagulation time, bleeding time, clot retraction, prothrombin time (one stage), complete blood count including platelet count and capillary fragility (tourniquet test). Since diagnosis, choice of therapy and prognosis depend upon the results of laboratory methods, meticulous attention to details is necessary in these preliminary tests as in the more elaborate procedures (See Table 18).

Coagulation Time Blood is obtained by vein puncture. Capillary coagulation tests are unreliable because blood obtained by skin puncture is mixed with tissue juice and therefore is unsuitable for determining the clotting time. If the vein puncture is not accomplished immediately and if there is any undue manipulation the procedure is repeated with another needle and vein. The Lee and White technique is used. One milliliter of blood is transferred into each of two clean dry Pyrex tubes measuring 13 by 100 mm. and tests are carried out in a water bath kept at 37° C. The first tube is tilted at half minute intervals until no blood flows when the tube is inverted. The second tube is then similarly tilted until the blood is solidified. The point at which the blood is solidified is designated as the coagulation time. The normal range of coagulation time is five to twelve minutes.

Bleeding Time Bleeding time is determined by the Duke method or by the Ivy method.

DUKE METHOD With a No. 11 Bard Parker blade a small cut is made in the ear lobe to a depth of 2 to 3 mm. With a round piece of filter paper the blood is blotted off not wiped off every thirty seconds. The blood should be permitted to flow onto the filter paper by capillarity. The interval between the time the incision is made and the moment when bleeding stops represents the end point. The range of normal is given as two to seven minutes falling between one and four minutes in the majority of patients.

Table 18 Results of Routine Tests in Hemorrhagic States*†

Disorder	Bleeding Time	Clotting Time	Clot Retraction	Tourniquet Test	Platelet Count	Prothrombin Time
Platelet abnormalities						
Thrombocytopenia	A	N	N	N	N	N
Thrombasthenia	A	N	N or N	N or N	N	N
VHC deficiency	N	A	N	N	N	N
PTC deficiency	N	A	N	N	N	N
PTA deficiency	N	A‡	N	N	N	N
Prothrombin deficiency	N	N	N	N	N	N
Labile factor deficiency	N	N§	N	N	N	A
Stable factor deficiency	N	N	N	N	N	N
Fibrinogen deficiency	N	N	N	N or A	N	N or A
Vascular disorders	N	N	N	N or A	N	N
Stuart Prower factor deficiency	N	N	N	N	N	A

From Schulman J and Smith C H: Coagulation Disorders in Infancy and Childhood. In Levine S Z (editor): Advances in Pediatrics, vol 9. Chicago, 1957. Year Book Publishers Inc. p 231.

†N = normal; A = abnormal.

‡May be normal in mild to moderate degrees of deficiency.

§May be prolonged with marked deficiency.

|| Abnormal with marked deficiency since no endpoint present.

Puncture of the ear lobe should be avoided in children with suspected hemophilia, pseudohemophilia, and severe thrombocytopenic purpura because bleeding may be so uncontrollable as to eventually require transfusions for hemostasis.

IVY METHOD. A blood pressure cuff placed just above the bend of the elbow is kept at 40 mm pressure. The surface of the forearm is cleansed with an antiseptic and then dried. With a Bard Parker blade or a sterile spring lancet the skin is punctured to a depth of 2 to 3 mm and a width of 2 mm in the fleshy part of the forearm below the elbow. The drops of blood are removed with a piece of filter paper every thirty seconds as is done in the Duke method. The bleeding time is the time which elapses between the puncture of the forearm and the cessation of bleeding.

This method has been recommended in children because persistent bleeding from the forearm can be more readily controlled by pressure than that from the ear lobe. Despite the possibility of prolonged oozing, the Duke test is still in current use and has been found reliable in most laboratories.

Clot Retraction. The clot in one of the tubes used in determining the clotting time is used for the clot retraction test. The test should be facilitated by loosening the clot from the walls of the tube with a platinum wire or glass applicator. The tube is stoppered, placed in a water bath at 37° C, and inspected at one, two, twelve, and twenty-four hours. Normal clot retraction is usually complete in one or two hours.

In the blood of patients with abnormal conditions contractility is minimal or absent after twenty four hours. Clot retraction depends upon several factors: the number of platelets, the concentration of fibrinogen and the cell volume. Adequate numbers of intact platelets are essential for clot retraction; the higher the concentration of fibrinogen, the less the contraction. Poor clot retraction is noticeable with counts below 80,000 per cubic millimeter. No retraction is observed in patients with thrombocytopenia with counts below 20,000 per cubic millimeter and in those with thrombasthenia (normal number of platelets but impaired function). It is poor in patients with an excessive red cell mass. Clot retraction is difficult to measure quantitatively although tests are available for that purpose.

Platelet Count The reagent used is 3.8 per cent sodium citrate solution. For the Rees-Ecker modification brilliant cresyl blue is added. An accurate platelet count is a prerequisite for the diagnosis of either thrombocytopenia or thrombocytosis. Thrombocythemia refers to more persistently increased platelet levels than are observed in patients with thrombocytosis after splenectomy. It is commonly seen in patients with myeloproliferative conditions such as chronic myelocytic leukemia, Hodgkin's disease and Boeck's sarcoid. Purpuric bleeding often with a prolonged bleeding time has been reported in patients with an excessively high platelet count. This situation has been encountered most often in patients with polycythemia vera, chronic myeloid leukemia and myeloid metaplasia.¹⁰ In those with thrombocytopenic purpura the blood smear presents a transparent appearance under the microscope due to reduced platelet numbers. As in patients with many conditions which are accompanied by thrombocytopenia, variations in morphology are likely to occur.

Syndromes have been described in which abnormal bleeding has been attributed to qualitative platelet defects—the so-called thrombocytopathic purpuras or thrombasthenia. The platelets are usually normal, rarely slightly reduced in number, highly abnormal in appearance, giant-sized, abnormally small, bizarre in shape, and agranular. Glanzmann's thrombasthenia¹¹ is cited as an example of a syndrome in which hemorrhage occurs with a normal bleeding time, platelet count and coagulation time but with poor clot retraction and abnormal platelet morphology. With sharper separation of platelet functions it may be possible eventually to relate a bleeding disorder to a specific defect. Patients have already been described in whom there is a defect in thromboplastin formation, clot retraction and antiheparin activity of the blood—functions of platelets necessary for maintenance of normal hemostasis.^{11a} Treatment of these patients is limited to transfusions of fresh or whole blood or platelet-rich plasma.

Tourniquet (Rumpel-Leede) Test The tourniquet test serves as a measure of capillary resistance. It consists of obstructing venous blood flow by a constricting band on the arm (blood pressure cuff) and recording the number of petechiae in a specified area distal to the obstruction. The intracapillary pressure is increased sufficiently to demonstrate a state of latent increased permeability (capillary fragility). The test is commonly performed by maintaining the blood pressure midway between the systolic and diastolic pressures for three to five minutes. After the cuff is removed the number of petechiae in a previously marked circular

area 5 cm in diameter on the flexor surface of the forearm a little below the bend of the elbow is counted. A count above five is interpreted as positive. Although the capillary resistance tends to parallel other manifestations of vascular inadequacy many exceptions are encountered so that a positive test is not always observed with a prolonged bleeding time and thrombocytopenia.

Significance of Routine Coagulation Tests The screening tests thus far described provide unequivocal evidence of a single defect—namely, an abnormality of platelets. The combined abnormalities of clot retraction, bleeding time, the tourniquet test and of course a low platelet count support such a diagnosis.

Markedly prolonged coagulation time is most commonly found in patients with deficiencies of the plasma thromboplastin precursors (AHG, PTC and PTA) with severe fibrinogen deficiency and with circulating anticoagulants. Mild degrees of AHG, PTC and PTA deficiencies may coexist with entirely normal whole blood clotting time. Abnormal results of the routine tests indicate the existence of a hemorrhagic state but do not give sufficient information for a specific diagnosis. On the other hand, normal results with these tests do not exclude a coagulation defect, especially when the history is suggestive. Further investigations carried out systematically are therefore necessary to detect minor degrees of depletion.

Tests for Phase I of Coagulation

The tests for the first phase of coagulation are primarily designed to differentiate the three types of hemophilia: classic hemophilia (hemophilia A) due to AHG deficiency, Christmas disease (hemophilia B) due to PTC deficiency, and PTA deficiency (hemophilia C). Platelet defects in number or quality can also be recognized by these tests.

In patients with each type of hemophilia regardless of severity, the platelet count, bleeding time, clot retraction, prothrombin time and fibrinogen content are normal. The severe grades of the hemophilias are characterized by markedly prolonged coagulation time. In patients with milder types, however, the clotting time may be only slightly prolonged and in those with the mildest types it may be entirely normal. With more elaborate and sensitive tests it is possible to detect lesser degrees of deficiency of the three plasma proteins (AHG, PTC and PTA).

The tests currently employed in first phase coagulation abnormalities are the thromboplastin generation test and the prothrombin consumption test.

Thromboplastin Generation Test Although the prothrombin consumption test detects moderate deficiencies of thromboplastin, it is normal in patients with mild deficiencies of the factors (AHG, PTC or PTA) that enter into its formation. The thromboplastin generation test described by Biggs and Douglas¹⁸ has proved to be a more sensitive test of thromboplastin formation defects. It not only permits the detection of slight degrees of abnormality but more important, it differentiates one type of abnormality from another, a distinction not provided by the whole blood clotting time or prothrombin consumption test. Specific deficiencies of antihemophilic globulin, plasma thromboplastin component and plasma thromboplastin antecedent can thus be ascertained. When

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Table 19 Constituents of Reagents* Prepared From Normal Blood*

Reagent	Phase 1	Phase 2	Phase 3
Whole plasma†	AHG ITC ITA ST P factor	1 r thrombin Labile factor Stable factor ST P factor	Fibrinogen
Adsorbed plasma	AHG PTA	Labile factor	Fibrinogen
Serum	ITC ITA ST P factor	Stable factor ST P factor	—

Modified from Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers p 231

While labile factor appears in whole and adsorbed plasma and influences the thromboplastin generation tests it is usually assayed in Phase 2 Stuart Prower factor deficiency also gives Phase 1 and 2 abnormalities and may be assayed in both phases However in Phase 2 it is separated from stable factor deficiency by the Stypven test mentioned in the text

Table 20 Effects of Normal Plasma Normal Deprothrombinized Plasma and Normal Serum on the Coagulation Abnormalities in AHG PTC and PTA Deficiencies

Reagent added to Latent plasma	Deficiency		
	AHG	PTC	PTA
Normal plasma	Corrects	Corrects	Corrects
Normal adsorbed plasma	Corrects	No correction	Correct
Normal serum	No correction	Corrects	Corrects

From Schulman I and Smith C H Coagulation Disorders in Infancy and Childhood In Levine S Z (editor) Advances in Pediatrics vol 9 Chicago 1957 Year Book Publishers Inc p 231

tests in which plasma is used 0.025M calcium chloride is generally employed The factors present in each of these preparations are given in Table 19

Platelets in Thromboplastin Generation Test—Thrombasthenia and Thrombopathia 1106a 110b It has been taken for granted that the platelets are normal ac

carried out in patients with disease of varying clinical severity the amount of thromboplastin formed bears some relation to clinical severity

With this test the progressive activity of plasma thromboplastin is evaluated by incubating at 37° C a mixture of adsorbed barium sulfate plasma (normally containing antihemophilic globulin AHG and plasma thromboplastin antecedent PTA) serum (normally containing plasma thromboplastin component PTC plasma thromboplastin antecedent PTA and Stuart Prower factor) platelets (or substitute) and calcium chloride At regular intervals measured samples of the incubation mixture are added to normal plasma (substrate) The thromboplastin generated in this incubation mixture causes clotting of the plasma and the speed of clotting is a measure of thromboplastin formation When all the first stage coagulation factors are present in normal amounts, the thromboplastin produced by the generating mixture after three to seven minutes incubation is sufficiently potent to achieve a plasma clotting time of eight to ten seconds

The thromboplastin generation test is similar to the prothrombin time inasmuch as both utilize the property of thromboplastin to shorten the clotting time of recalcified plasma

Preparation of Coagulation Factors for Thromboplastin Generation Test Except for fibrinogen which can be measured quantitatively the clotting factors involved in the production of thromboplastin and of the accessory factors in prothrombin conversion can be identified by analyzing their distinctive behavior and interaction in especially devised clotting systems With the use of minimal suspensions which selectively adsorb some factors in plasma and not others the knowledge that certain factors are present in plasma and absent in serum and the knowledge of the effect of storage upon plasma and serum factors it is possible to determine the presence or absence of factors in each stage of coagulation In testing for deficiencies in the first stage advantage is taken of the fact that adsorption removes several factors from normal plasma but leaves AHG and factor V (labile factor) intact

PLASMA In many of the coagulation studies plasma rather than whole blood is used It is prepared with one of the anticoagulants most frequently sodium oxalate When obtained from normal blood plasma contains all the coagulation factors in each phase of coagulation

ADSORBED PLASMA By treating the oxalated plasma with tricalcium phosphate gel or barium sulfate deprothrombinized plasma results These adsorbing agents remove not only prothrombin but also PTC stable factor and Stuart Prower factor (ST P) leaving AHG PTA labile factor and fibrinogen For citrated blood aluminum hydroxide is used as the adsorbing reagent

SERUM If normal whole blood is allowed to clot in a test tube antihemophilic globulin (AHG) labile factor prothrombin and fibrinogen are consumed Normal serum then contains PTC PTA stable factor and Stuart Prower factor (ST P) These serum factors are stable when stored

PLATELETS In many of the tests performed with these blood products suspensions of normal platelets are required More recently platelet substitutes such as human brain or soy bean cephalin have been used

CALCIUM CHLORIDE Calcium chloride is required to institute clotting in all

and values of more than 15 to 20 per cent to result in a normal thromboplastin generation. Hence abnormal tests will result only when the antihemophilic content of plasma falls below these levels.

Prothrombin Consumption Test The prothrombin consumption test is performed on serum after clotting has occurred in a manner similar to the one stage test for prothrombin time of plasma with one addition. Since serum contains stable factor but not fibrinogen or labile factor these must be supplemented in order to determine the residual prothrombin after clotting. The additional factors required are present in plasma which has been adsorbed with barium sulfate or tricalcium phosphate. The treated plasma contains labile factor and fibrinogen but it is free of prothrombin.

With a 1 ml serologic pipette 0.1 ml of serum to be analyzed (residual after clotting) is blown into a mixture of 0.1 ml of calcium chloride 0.1 ml of adsorbed plasma (source of labile factor and fibrinogen) and 0.1 ml of thromboplastin reagent (as in the test for plasma prothrombin time). The time required for the clot to form is accurately measured.

The normal prothrombin consumption time is over twenty five seconds⁴⁴ thus indicating very little residual prothrombin. The usual range of residual prothrombin in serum is given as 0 to 25 per cent. Usually 75 per cent is converted to thrombin. In patients with disorders of phase 1 the serum prothrombin time is below twenty five seconds and in severely affected patients as short as twelve to fourteen seconds. In principle the prothrombin consumption test is similar to the Quick prothrombin time test inasmuch as the former measures the prothrombin remaining in serum and the latter that which is present in the plasma before coagulation.

Interpretation of Prothrombin Consumption Test The prothrombin consumption test is a quantitative determination of the residual prothrombin in serum after clotting has occurred. When normal blood in a test tube clots sufficient thromboplastin is formed in the initial phase of coagulation to convert virtually all of the prothrombin to thrombin so that at completion of the process there is little or no prothrombin left in the serum. If all the prothrombin is utilized and little or none is left in the serum it can be taken for granted that adequate amounts of thromboplastin have been formed and that this first phase of coagulation is relatively normal. On the other hand abnormalities of this phase are indicated by the presence of a large amount of residual prothrombin in the serum after clotting, indicating the formation of inadequate amounts of thromboplastin required to convert prothrombin to thrombin. Prothrombin activity of the serum is determined one hour after coagulation since thrombin continues to be formed after the blood or plasma has clotted. After this interval practically all the prothrombin has been consumed. The prothrombin consumption test though useful in detecting defects affecting the formation of thromboplastin is less sensitive than the thromboplastin generation test.

Tests for Phase 2 of Coagulation

Prothrombin Complex Deficiencies In carrying out *in vitro* tests prothrombin factor V, factor VII and the Stuart Prower factor must be present in sufficient

according to the screening tests thus having been previously established by normal platelet count, clot retraction, bleeding time and tourniquet test. However, in patients with platelet dysfunction the qualitative deficiency will be reflected in an abnormal thromboplastin generation test when the patient's platelets are placed in a system with normal adsorbed plasma and normal serum. The test is corrected with normal platelets or platelet substitutes.

The terms thrombasthenia and thrombopathia are often applied interchangeably to conditions in which platelets are qualitatively defective although normal in numbers. In both conditions there is a severe bleeding tendency. Thrombasthenia refers to a defective function of platelets in clot retraction; thrombopathia (thrombocytopathia) refers to a defect in platelets in thromboplastin formation. Thrombasthenia has been further characterized as an inability of platelets to form pseudopods and a lack of spreading in plasma and serum, resulting in a disturbance in clot retraction.³⁰ In thrombocytopathia, pseudopod formation and spread are normal but the platelets are giant in size and thromboplastin generation and prothrombin consumption are disturbed. Thrombopathia has often been used as an all inclusive term for any qualitative platelet defect.

The thromboplastin generation test can be modified to provide quantitative estimates of AHG activity and of other hemophilic factors.³¹

A rapid screening test for thromboplastin generation has recently been reported by Hicks and Pitney.³² It is a useful and rapid method for eliminating those specimens in which further investigation is unnecessary. The principle of the test is the recalcification of diluted whole plasma in the presence of a platelet substitute. Thromboplastin generation is determined by adding samples of the incubation mixture to high speed normal recalcified plasma.

Mutual Correction Tests The various hemophilias may be differentiated simply by testing for mutual correction in mixtures of blood or plasma from the patient with the unknown defect and from patients whose defects have been identified. Blood or plasma from a patient with AHG deficiency will correct abnormal coagulation in a test tube of blood from patients with plasma thromboplastin component and plasma thromboplastin antecedent defects but not from another with AHG deficiency. The same principle applies for correction of PTA deficient blood and plasma by AHG or PTC but not by PTA samples and for the correction of PTC deficient blood by AHG and PTA but not by PTC sample. The difficulties in mutual correction tests are that a panel of blood samples from patients with known deficiencies is not always available; hence the advantage of the precise diagnostic facilities of the thromboplastin generation test.

Comparative Value of Laboratory Tests in Detecting Thromboplastin Deficiency Antihemophilic globulin is normally present in plasma in a range of 50 to 170 per cent. Intermediate degrees of bleeding occur between the almost negligible levels of AHG in patients with classic hemophilia and the lower limit of normal. The variable sensitivity of the tests enumerated for phase 1 abnormalities must therefore be considered in the diagnosis of hemophilia. Thus as little as 1 per cent antihemophilic globulin is sufficient to produce a normal whole blood clotting time, 3 to 5 per cent to yield a normal prothrombin consumption

prothrombin time by the addition of serum the patient lacks stable factor; if there is no improvement by adding both reagents (deprothrombinized plasma and serum) the patient has a deficiency of prothrombin itself.

Normal plasma, serum, and adsorbed plasma (with barium sulfate hence deprothrombinized) contain the following factors of the prothrombin complex: normal plasma—prothrombin and labile stable and Stuart Prower factors; normal serum—stable factor and Stuart Prower factor; and adsorbed plasma—labile factor.

The differentiation of the prothrombin complex deficiencies is indicated in Table 21. Not included in the table is Stuart Prower factor deficiency which follows the same pattern as stable factor (factor VII) deficiency.

Table 21 Differentiation of Prothrombin Complex Deficiencies

Deficient Factor Causing Prolonged Prothrombin Time	Effect of Addition of		
	Normal Plasma	Serum	Adsorbed Plasma
Prothrombin	Corrects	No correction	No correction
Labile factor	Corrects	No correction	Corrects
Stable factor	Corrects	Corrects	No correction

Other differential tests for labile factor and stable factor deficiencies are based on the fact that when oxalated plasma is stored labile factor disappears rapidly, whereas stable factor and prothrombin remain in high concentration. A reagent for determining the stable factor deficiency can also be prepared by the passage of plasma through a 30 per cent asbestos filter which removes stable factor but retains prothrombin and labile factor.

Combined Prothrombin and Stable Factor Deficiency If the patient's plasma does not correct the prolonged prothrombin time with aged plasma (contains stable factor but no labile factor) labile factor is deficient. If labile factor is present and the addition of small amounts of serum (contains abundant stable factor) only partially corrects the prothrombin time then presumably both prothrombin and stable factor are deficient.

Stuart Prower Factor Investigation of patients with a long Quick prothrombin time led to the discovery of a defect heretofore ascribed to a deficiency of factor VII.^{16, 17} The Stuart Prower factor is present in normal plasma and is relatively heat and storage stable. This factor is now known to be essential both for normal plasma thromboplastin production and conversion of prothrombin to thrombin by its interaction with tissue extract. The Stuart Prower factor and factor VII are both adsorbed by tricalcium phosphate and other similar adsorbing agents. A deficiency of the Stuart Prower factor results in an abnormal thromboplastin generation test. It can be differentiated from factor VII when Stypven

amounts to convert prothrombin to thrombin and give a normal prothrombin time. These factors collectively are frequently designated as the prothrombin complex. A failure to effect conversion to thrombin may result from a deficiency of one or a combination of these factors. It should again be emphasized that these are *in vitro* tests designed to isolate defects of any one or a combination of factors in the laboratory.

Prothrombin Time (Plasma Prothrombin Time) The one stage test of Quick⁹³ has been found most useful in determining disturbances of the second phase of coagulation (the conversion of prothrombin to thrombin after activation by thromboplastin). By adding an excess of tissue extract (thromboplastin) and optimal amounts of calcium to measured samples of citrated or oxalated plasma, prothrombin activity is measured by the time required for coagulation to occur. The one stage prothrombin time serves as a specific indicator of phase 2 involvement and includes the entire prothrombin complex—prothrombin and the accelerator agents labile, stable and Stuart Prower factors. In our laboratory the normal prothrombin time by this method is eleven to fifteen seconds. It should be remembered that no definitive prothrombin time can be stated which is the same for every laboratory. Since the value obtained depends upon the potency of the thromboplastin used, a normal control must be simultaneously determined. In terms of prothrombin activity by comparison on a curve from suitable plasma dilutions, the normal range is from 70 to 120 per cent.

An elevated prothrombin time may reflect any single or combined deficiency of prothrombin, factor VII, factor V, Stuart Prower factor, and fibrinogen (absence of end point). An unusually low plasma content of fibrinogen may also account for a prolonged one stage reading, but this value can be determined chemically. A normal prothrombin time serves therefore incidentally as evidence of a normal fibrinogen content of plasma. With the help of simple correction tests, a deficiency of either labile or stable factor can be readily determined.

The one stage prothrombin test is performed as follows. The thromboplastin used is generally obtained from rabbit brain or rabbit lung. Many excellent, well standardized thromboplastin preparations are commercially available. This test requires exacting technique; the patient's plasma must be obtained in a standard manner, the concentration of calcium chloride must be exact, the temperature must be controlled at 37° C., a stop watch is used for timing, and controls are carried out. With most techniques 0.1 ml. of thromboplastin is then added. Timing is started at this point and the clotting time is recorded. Commercial preparations are available in which the thromboplastin and calcium are already mixed in proper amounts (Simplastin). The test then requires only one step. Preparations of control plasma for testing thromboplastic reagent are also commercially available (Diagnostic Plasma, Protolab). As previously stated, with most techniques the one stage prothrombin times are usually twelve to fourteen seconds.

Differential tests can be carried out in conjunction with the one stage prothrombin time to determine a deficiency of factor V, factor VII, prothrombin, or Stuart Prower factor. If there is correction of the Quick prothrombin time by the addition of the patient's plasma to deprothrombinized plasma (barium sulfate-absorbed), the patient lacks labile factor; if there is correction of the Quick

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(Russell's viper venom) combined with cephalin replaces tissue thromboplastin in the prothrombin test. An abnormal prothrombin time is then obtained with Stuart Prower factor deficiency in contrast to a normal prothrombin time with plasma deficient in factor VII. Factor VII is not needed in the thromboplastin generation test whereas the Stuart Prower factor is a necessary ingredient. The clotting time in Stuart Prower factor deficiency is slightly prolonged. Stuart Prower levels were found to be low in premature newborn infants at birth with an increase toward normal by the sixth day of life whether or not vitamin K₁ was given to the infants at birth.¹⁰⁶

Tests for Phase 3 of Coagulation

Fibrinogen Deficiencies With adequate thrombin formation fibrinogen is converted to solid fibrin. Fibrinogen occurs in plasma in a concentration of approximately 250 to 400 mg per 100 ml. A threshold concentration of at least 60 mg per 100 ml is necessary for clotting to be detectable.¹¹ Many methods are available for clinical quantitation of fibrinogen after its separation from plasma. One of the simplest methods for clinical use is to measure the turbidity of a suspension of salt precipitated fibrinogen with a spectrophotometer.⁴⁶ A simple test for determining the presence of fibrinogen in coagulable blood is the addition of thromboplastin or thrombin. In patients with fibrinogenopenia the blood will remain incoagulable.

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The Purpuras

The purpuras embrace a miscellaneous group of blood disorders which have in common skin hemorrhages with or without bleeding into the mucous membranes and other sites and into internal organs. Extravasations of blood may vary from small pinpoint petechiae to large ecchymotic areas. Purpura is a variable symptom complex resulting from a diversity of etiologic agents often with thrombocytopenia and with other conditions in which there are no platelet changes but which have striking clinical features. Purpuras can therefore be classified as thrombocytopenic in which case they are classified as either idiopathic or secondary to well defined disorders or as nonthrombopenic such as Henoch Schonlein disease.

THROMBOCYTOPENIC PURPURAS

Following are the thrombocytopenic purpuras due to either quantitative or qualitative platelet abnormalities

- 1 With low platelet count (thrombocytopenic states)
 - A Idiopathic thrombocytopenic purpura
 - B Symptomatic thrombocytopenic purpura
 - (1) Caused by infections
 - (a) Sepsis
 - (b) Subacute bacterial endocarditis
 - (c) Typhus
 - (d) Measles
 - (e) German measles
 - (f) Scarlet fever
 - (g) Hemorrhagic smallpox
 - (2) Caused by chemicals, drugs, physical agents (x rays, radioactive substance)
 - (3) Associated with blood disorders
 - (a) Secondary to marrow infiltration: leukemia, neoplasm, fibrosis
 - (b) Anemias (hypoplastic and aplastic) in association with autoimmune hemolytic anemia, pernicious anemia, megaloblastic anemia of infancy
 - (c) Secondary to splenic hyperfunction in various diseases—Gaucher's disease, Banti's syndrome, Felty's syndrome, lymphomas, lupus erythematosus, hypersplenism
 - (d) Miscellaneous blood disorders—congenital and neonatal thrombocytopenia, thrombocytopenia associated with hemangioma, thrombotic (thrombohemolytic) thrombocytopenic purpura, infectious mononucleosis, massive blood transfusions

- With normal platelet count
 - A Allergic purpura
 - Congenital vascular defects
 - C Purpura simplex
 - D Idiopathic pulmonary hemosiderosis
 - E Infections such as meningococcemia (Waterhouse-Friedrichsen syndrome) and scarlet fever (purpura fulminans)
 - F Drugs and chemical agents and avitaminosis (scurvy)
 - G Thrombasthenia and thrombophilia

Thrombocytopenic states may also be classified on the basis of the bone marrow content of megakaryocytes⁸⁴

- 1 With normal or increased megakaryocytes in the marrow
 - A Idiopathic thrombocytopenic purpura
 - B Congenital (neonatal) thrombocytopenic purpura
 - C Secondary to splenic hyperfunction in various diseases (Gaucher's disease, Banti's syndrome, Ichthyosis syndrome, lymphomas, lupus, hypersplenism)
 - D Drug sensitization (such as caused by quinidine)
- 2 With decreased or absent megakaryocytes in the marrow
 - A Secondary to marrow replacement by tumor, leukemia, fibrosis
 - B Aplastic anemia
 - C Marrow damage due to drugs, chemical agents (such as chloramphenicol), radiation, etc.
 - D Congenital (neonatal) thrombocytopenic purpura

In patients with amegakaryocytic thrombocytopenia the megakaryocytes are strikingly reduced in number. Most often the disappearance of megakaryocytes is due to replacement and filtration of bone marrow by abnormal cells, as occurs in patients with leukemia and lymphosarcoma and other types of neoplasms. It will be noted that congenital thrombocytopenia may be associated with either decreased or normal numbers of megakaryocytes.

Idiopathic Thrombocytopenic Purpura (ITP, Werlhof's Disease, Purpura Hemorrhagica)

Idiopathic thrombocytopenic purpura is a disease of unknown etiology characterized by a hemorrhagic tendency resulting from a marked reduction in the number of platelets with extravasation of blood into the skin, mucous membranes and subcutaneous tissues.

Pathogenesis. Many theories have been suggested for the pathogenesis of idiopathic thrombocytopenic purpura to explain its central features: the thrombocytopenia, increased capillary permeability, and the role of the spleen.¹⁰ These include the movement of platelets from the circulation to the site of injured vessels with corresponding reduction in the peripheral blood;⁶⁷ decreased platelet formation due to an abnormal humoral factor elaborated by the spleen and inhibiting megakaryocytic function¹¹ and the destruction of platelets after their selective sequestration by the spleen.⁶

More recently interest has shifted to an immunoallergic mechanism as an etiologic factor. Information from two sources suggested the possibility that an immunologic reaction might be involved in idiopathic thrombocytopenic purpura. The observation that infants born to mothers with this disease were often pur-

puric was ascribed to the transmission of an immune body across the placenta. In addition Evans and co workers^{8,9} presented evidence for the existence of a relationship between acquired hemolytic anemia and primary thrombocytopenic purpura. They noted that acquired hemolytic anemia with sensitization of the red cells is often accompanied by thrombocytopenia and that primary thrombocytopenia in turn was frequently accompanied by red cell sensitization with or without hemolytic anemia. Since acquired hemolytic anemia had been shown to be due to an autoantibody Evans and associates suggested that primary thrombocytopenic purpura was due to a thrombocyte autoantibody.

Harrington and co workers¹⁷ have demonstrated that an immunologic mechanism is responsible for the low platelet count in many patients with idiopathic thrombocytopenic purpura. Platelet agglutinins have been demonstrated in vitro in the plasma of many patients whose thrombocytopenia was of the idiopathic variety. A factor presumably identical to this platelet agglutinin is capable of inducing thrombocytopenic purpura and altering megakaryocytes in normal recipients of this plasma. In terms of this concept the spleen is involved in pathogenesis by removing sensitized platelets and producing platelet agglutinin in variable amounts. Some of the antibody is made in the spleen, the major portion is made elsewhere.

In spite of the evidence that idiopathic thrombocytopenic purpura is based on an immunologic mechanism circulating platelet antibodies have not been universally found with standard procedures.

That a capillary defect undoubtedly plays a role in the etiology of the disease is reflected in the prolonged bleeding time and purpuric manifestations. On the other hand there is no true correlation between the bleeding time or hemorrhage and the degree of thrombocytopenia. A factor in pathogenesis is the demonstration¹⁰ that normal capillary contraction does not occur in patients with thrombocytopenic purpura and other hemorrhagic states.

The concept of a "thrombopoietin" for platelet formation comparable to erythropoietin for red cells is suggested by a case report of a child with chronic idiopathic thrombocytopenic purpura in whom a marked increase in the number of platelets followed transfusion with fresh or stored blood or plasma.⁷⁴ The pathogenesis of the purpura in this patient appeared to be related to a congenital deficiency of a platelet stimulating factor present in normal plasma. The factor appears to act by stimulating megakaryocyte maturation and platelet production.¹¹ The existence of a human thrombopoietin system regulating thrombopoiesis was also described in thromboethemic human serum.¹⁵

Clinical Manifestations Idiopathic thrombocytopenic purpura occurs in one of two major types—an acute self limited form or a chronic protracted disease with occasional remissions. The disease occurs most commonly in children and young adults with the greater frequency in younger age groups and usually between 2 and 8 years of age.¹⁶ In the adult the disease occurs four to five times as frequently in females as in males; in children no sex difference is noted.

The acute form which is common in children has a sudden onset frequently after an upper respiratory infection or following measles,²⁴ rubella,^{1,30,37} chicken pox,^{31,3} mumps or infectious mononucleosis. It is usually difficult to relate the

disease to an infection or to a drug employed during treatment. An increased incidence of allergic manifestations has been found among relatives of patients and in about 15 per cent of the patients.¹

The chief complaint is easy bruising. Cutaneous purpura is either spontaneous or secondary to minor trauma. The size of the hemorrhage varies from small petechiae of pin head size to large ecchymoses. The initial period is often marked by widely scattered petechiae with purpura with large extravasations of blood following later. The color changes from red to purplish to brown with progressive liberation of pigments. Nosebleeds, gingival bleeding and bleeding into and from the oral mucous membranes, gastrointestinal tract, kidneys and vagina frequently accompany the purpura, especially at the onset. Petechiae may be found in the subconjunctivae and in the palate. The interior surfaces of the lower extremities, the buttocks and especially over bony prominences such as the ribs, scapulae, shoulders, legs and pubic area are commonly affected. Hematomas form over the lower extremities. Hematuria, hematemesis, melena and hemarthroses are infrequent. Menorrhagia at or shortly after puberty may be the first indication of idiopathic thrombocytopenic purpura with a deficiency in blood platelets and impaired capillary resistance appearing soon after.⁴⁰ Intracranial hemorrhage is uncommon in children and constitutes the most serious complication of this disease occurring usually early in its course. With the exception of purpuric manifestations there are few physical findings. The spleen is not palpable or is barely so. If significant splenomegaly is present other diagnoses should be considered, notably leukemia, lymphosarcoma, Hant's syndrome or other conditions associated with hypersplenism.

Laboratory Data. Examination of the bone marrow and peripheral blood are usually sufficient to establish the diagnosis.

Blood Findings. The most significant finding in the laboratory examination of the patient with thrombocytopenia is a platelet count usually below 60,000 per cubic millimeter and frequently below 20,000. This may be confirmed by the sparsity of platelets in the blood smear in which the relatively few platelets seen are often single, large and abnormal in shape. The bleeding time is characteristically prolonged, the tourniquet test is positive and clot retraction is poor to absent. The prothrombin time and whole blood clotting time are normal. The existence of a true disturbance in the coagulation mechanism in idiopathic thrombocytopenic purpura is demonstrated by an abnormal prothrombin consumption test which is indicative of impaired thromboplastin formation. A normochromic anemia with reticulocytosis occurs if there is epistaxis or if bleeding into the urinary tract or gastrointestinal canal is severe. A moderate leukocytosis with an increase in granulocyte forms occurs if purpura is excessive.

Bone Marrow Findings. In patients with idiopathic thrombocytopenic purpura the principal abnormality is confined to the megakaryocytes. In patients with active bleeding, normoblastic and often myeloid hyperplasia may be present. The megakaryocytes are normal or increased in number and show reduced platelet formation. The megakaryocytes vary in size, amount of cytoplasm and lobulation of the nucleus. Agranular vacuolated degenerated and immature forms are present.

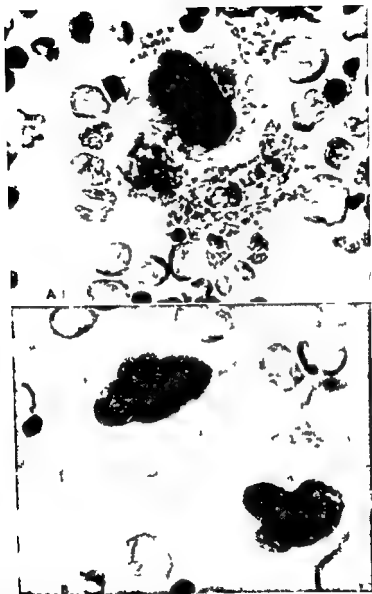


Fig 46 A Smear from normal bone marrow. Note mature megakaryocyte containing large lobulated nucleus. Cytoplasm is granular with platelets in the process of formation. Masses of platelets are chiefly grouped about the periphery of the cell. B Intermediate megakaryocytes. These are usually found in increased numbers in the bone marrow of patients with idiopathic thrombocytopenic purpura. Cytoplasm is more abundant and less granular than that shown in A, and platelet differentiation is either absent (upper cell) or slight (lower cell). (Courtesy Dr. Robert L. Rosenthal, New York, NY.)

In one study¹⁸ platelet production was found in only 8 to 19 per cent of all megakaryocytes and following splenectomy in 69 to 85 per cent of all cells. As the disease enters the chronic phase megakaryocytes although more mature still show a reduction in granulation and platelet formation. The massive liberation of platelets after splenectomy has been interpreted as an indication that the spleen inhibits platelet formation from the megakaryocyte and prevents their delivery in the circulating blood.²⁰

Despite these considerations bone marrow examination cannot be regarded as diagnostic of idiopathic thrombocytopenic purpura. Its chief function lies in the exclusion of disease with which idiopathic thrombocytopenic purpura can be confused principally aplastic anemia and leukemia and less often lymphosarcoma and other infiltrative diseases.

Several studies have shown that there is no consistent pattern of megakaryocytes that can be correlated with the course or prognosis of idiopathic thrombocytopenic purpura.¹⁰ In patients with a complete or almost complete absence of megakaryocytes however splenectomy is contraindicated and an underlying condition should be sought. Bone marrow examinations should be repeated to establish the existence of decreased megakaryocytes. Eosinophilia in the bone marrow frequently observed in this disease and regarded as a favorable prognostic sign for spontaneous recovery¹⁰ has not been confirmed.

Diagnosis. It is important initially to distinguish between nonthrombocytopenic and thrombocytopenic purpura. To establish a diagnosis of idiopathic thrombocytopenic purpura secondary causative factors such as chemical and physical agents blood disorders and infections which depress platelets must be eliminated. Bone marrow aspiration is of aid in differentiating idiopathic thrombocytopenia from aplastic anemia and leukemia since it may resemble these conditions closely in some phase of the respective diseases. In patients with thrombocytopenia megakaryocytes are normal or increased and red cell and granulocytic precursors are normal. In those with aplastic anemia and leukemia all elements including the megakaryocytes are depressed (amegakaryocytic) and in the latter the bone marrow is infiltrated with blast forms. The thrombocytopenia of metastatic carcinoma and lymphosarcoma is also associated with an amegakaryocytic bone marrow.

In patients with idiopathic thrombocytopenia the spleen is not enlarged. Where moderate or marked splenomegaly exists in association with thrombocytopenia other conditions such as Gaucher's disease, Banti's syndrome and reticuloendotheliosis are to be considered. Sudden epistaxis or gastrointestinal hemorrhage together with thrombocytopenia in an infant whose spleen has become enlarged may indicate reticuloendotheliosis.

Disseminated lupus erythematosus may exist as an underlying disturbance manifesting itself solely as thrombocytopenic purpura without rash or joint manifestations. Examination for LE cells has therefore been recommended as a routine test in all patients with thrombocytopenic purpura preferably before steroids are administered. The development of overt disseminated lupus erythematosus following splenectomy for idiopathic thrombocytopenic purpura has been interpreted as an indication that the intact spleen exerts an inhibitory influence

in preventing the full expression of the latent disease¹⁹ Although the development of lupus erythematosus has been estimated to have occurred in as many as 25 per cent of all patients splenectomized for thrombocytopenic purpura²⁰ this sequence has not as yet been observed in our clinic nor in another children's clinic²¹

Course and Prognosis A spontaneous remission occurs in 75 per cent of infants and children as compared with 25 to 30 per cent of adults. The disease may run its course in a few weeks without recurrence. The majority of children recover completely within three months usually in the first six weeks and 10 to 15 per cent recover within four to six months. Spontaneous and permanent recovery may not occur for six months to one year during which time purpura remits and relapses with varying degrees of thrombocytopenia with each attack. About 10 per cent of children develop the chronic form and of these approximately 85 per cent recover with splenectomy. In chronically affected patients particularly a constant search must be made for an underlying disease particularly aplastic anemia in which the definitive features of the complete disease may not be manifest for a year or more.

Treatment Fresh blood platelet rich plasma platelet transfusions corticosteroids and splenectomy are used in the treatment of thrombocytopenic purpura. Early treatment consists of transfusions of fresh whole blood in the event of severe anemia from blood loss and the administration of adrenocortical steroids and less commonly ACTH. Although intracranial hemorrhage is rare in children rest or modified activity is advisable when platelet counts drop below 30 000 per cubic millimeter. Subsequent attendance at school and resumption of a normal regime depend upon cessation of cutaneous hemorrhages and an increase in the number of platelets.

Transfusions In the nonanemic patient whose bleeding is not controlled by steroids and tends to be progressive viable platelets can be obtained either as platelet rich plasma by sedimentation or slow centrifugation of fresh whole blood or as platelet concentrates obtained from several units of blood. Despite the natural tendency of the acute disease in most children to run a favorable course with a spontaneous remission the possibility of cerebral hemorrhage or other occult internal bleeding justifies the administration of steroids from the onset of the illness.¹⁰⁰ It has also been shown that nearly all deaths occur in the first month of the disease.⁴ Furthermore it is possible that steroids may shorten the disease by accelerating the onset of a remission.

Steroids As to the choice of a drug adrenocortical steroids and ACTH are both effective in the relief of bleeding symptoms both by an early hemostatic effect mediated by action on the blood vessel wall²² and by a later rise in platelets. Of the various agents prednisone or like substance is most useful because of ease of administration and the fact that it lends itself more readily to regulation of dosage. An increase in the number of platelets in the patient with acute idiopathic thrombocytopenic purpura with a favorable prognosis occurs within a week or ten days after the initiation of therapy. The mechanism of the platelet increase is not apparent one of the explanations offered is that prednisone re-

duces the concentration of antibody responsible for platelet destruction or megakaryocytic inhibition."

The dosage of steroids is variable and is adjusted for the age of the patient, severity of the disease and the individual tendency to develop the side effects. The amount of prednisone is similar to that given for acute leukemia (see chapter 23)—namely 15 to 20 mg daily for patients under 2 years of age and 20 to 60 mg for older children depending upon their age. For triamcinolone and methylprednisolone compounds four fifths of this dose is given (see Chapter 23). Like all adrenocortical steroids the drug is given in four divided doses at six hour intervals. In the infant the dose must be carefully adjusted to avoid hypertension even in this age period.

In the older child approaching adolescence or in the very acutely ill younger child it may be necessary to increase the dosage to 80 mg daily to obtain a favorable response. Usually a maximal dose of 60 mg daily is given in the first week of illness and is reduced to 40 mg for the remainder of the period of treatment.

Frequently a normal platelet level is obtained with three weeks of steroid treatment in which case the drug is tapered off during the fourth week. In the majority of patients only one course of treatment is required. Regardless of recovery blood counts are carried out at regular intervals for several months and years to assure the permanency of the remission. Maintenance therapy with smaller doses (2.5 to 15 mg per day) has been advocated when the platelets have reached normal levels¹⁻⁶ in the hope that this will assure a permanent remission and reduce the need for later splenectomy. This plan may be followed in children with a history of relapsing purpura in whom splenectomy seems inevitable. In the few patients in whom this form of treatment has been carried out the minimal dosage necessary to maintain adequate platelet response was 15 mg. There are several objections to maintenance therapy in children as a routine procedure: persistent and excessive hypercorticism, generalized osteoporosis and retardation of growth. If the response with steroids in successive courses is transient or inadequate better results are occasionally obtained by the intramuscular administration of three or four single daily doses of 60 units of corticotrophin in a gelatin menstruum. In an occasional patient after several courses of treatment with corticosteroids the platelet count may become stabilized at levels of approximately 100,000 per cubic millimeter without maintenance therapy and without evidence of purpura. Under these conditions splenectomy may be postponed.

Splenectomy Splenectomy was introduced as a form of treatment in this disease by Kiznelson.¹ Splenectomy is indicated if with adequate therapy thrombocytopenia persists for six months or longer especially when associated with recurrent purpura or overt bleeding provided secondary or symptomatic forms of thrombocytopenic purpura have been eliminated and megakaryocytes are present in normal or increased numbers in the bone marrow. The finding of a few or no megakaryocytes warrants a search for an underlying condition and is a contraindication to splenectomy. An expectant policy is followed beyond the six month period if the bleeding tendency is not manifested despite thrombocy-

topenia and the patient is asymptomatic. In such children a decision is usually reached when at the end of a year platelet levels either return to normal or are eventually associated with hemorrhagic manifestations. Complete clinical and hematologic remissions can be expected in about 85 per cent of children with the chronic disease in whom splenectomy is performed. In a series comprising patients of all ages splenectomy was successful in 81 per cent of those with idiopathic thrombocytopenic purpura whereas corticosteroid therapy was successful in only 38 per cent.¹

When splenectomy is contemplated preoperative preparation with steroids is useful in controlling hemostasis during the procedure.⁴ The therapeutic administration of the steroids is of equal or even greater importance in those patients who have had intermittent rather than continuous treatment. In the unprepared patient adrenal insufficiency may compromise the patient's ability to deal with acute stress which may develop during splenectomy. Hydrocortisone is preferable to prednisone or prednisone like compounds in the preoperative and operative periods. During childhood 50 mg. of hydrocortisone is given intramuscularly at twelve hour intervals on the day preceding operation and the blood pressure is observed. One dose is repeated in the morning of the operation and another is given during the operation by slow intravenous drip. It is a good plan to continue treatment for several days following the operation. Because of the excessive increase in the number of platelets in many of these patients postoperatively and because of the danger of thrombosis platelet levels should be followed routinely. Anticoagulant therapy is advised if platelet counts before the end of the first week postoperatively persist at levels over 1 million per cubic millimeter.⁴ The occasional susceptibility of the splenectomized child to overwhelming infection has prompted the routine use of a broad spectrum antibiotic for at least two years following the operation in our clinic.⁵

It should be pointed out that in certain children, especially those in the preadolescent and adolescent groups, splenectomy may not provide the anticipated cure. We have encountered several children in whom thrombocytopenia with a *tert* bleeding accompanied by a positive Coombs test with or without hemolytic anemia recurred following a remission of several months to a year. The failure of platelets to increase following splenectomy may indicate the presence of a more widespread disease and a fatal outcome. A case in point is that of a boy whose spleen was removed at 14 years of age for thrombocytopenic purpura. The platelet count following the operation remained depressed and could only be restored to normal levels by large doses of steroids. After a remission of nine months hemolytic anemia occurred in which the Coombs test was negative. Soon after recovery from the anemia marked thrombocytopenia recurred which could not be controlled and the patient eventually succumbed with a fatal hemorrhage. The Coombs test was positive in the final episode despite the absence of an anemia due to a hemolytic process. Throughout the entire course the test for lupus erythematosus was consistently negative. Such a combination of circumstances is suggestive of the concept of Evans and co-workers⁸⁻⁹ who emphasized the immunologic relationship between acquired hemolytic anemia and thrombocytopenic purpura.

Congenital Thrombocytopenic Purpura

Congenital thrombocytopenic purpura occurs infrequently, but the literature contains many case reports and studies bearing on its pathogenesis.⁶⁻⁸⁴ It appears in infants born of mothers with idiopathic thrombocytopenic purpura.

who either have had a splenectomy or are symptomless and unaware of a lowered content of platelets of normal mothers or of mothers with drug-induced thrombocytopenic purpura such as follows the ingestion of quinine.

Etiology Current concepts implicate an immunologic mechanism in the causation of the disease in the infant.¹ According to Harrington⁴ one third of the patients do not have any demonstrable autoantibodies for platelets and are assumed to have a defect of platelet formation from megakaryocytes as the sole factor causing thrombocytopenia. Mothers with this type of idiopathic thrombocytopenia give birth to normal infants. On the other hand patients with autoimmune idiopathic thrombocytopenic purpura possess a circulating antibody which damages both platelets and megakaryocytes. Mothers with this variety of thrombocytopenia give birth to infants with purpura. The antibodies which cross the placenta into the fetal circulation consist of autoagglutinins as well as isoagglutinins for the platelets of the mother and infant.

In the case of normal mothers it has been postulated that the mother develops isoagglutinins for the baby's platelets presumably on the basis of platelet incompatibility between mother and infant in a manner analogous to Rh sensitization in infants with erythroblastosis. She may also have been sensitized to platelet antigen in a previous transfusion or to fetal platelets of a different antigenic composition during pregnancy. The precise nature of the immune substance is not always obvious.

Quinine-induced thrombocytopenic purpura in a mother and her newborn infant has been observed⁶¹ with *in vitro* evidence of quinine platelet antibodies in the plasma of the mother and of the infant just after delivery. The administration of quinine before delivery resulted in thrombocytopenia and purpura due to the presence of preformed antibody from maternal sensitization to the drug originally given in childhood.

Prognosis There have been several families in which consecutive babies have had neonatal thrombocytopenic purpura. The prognosis in the infant is usually good with the restoration of a normal number of platelets within the first two or three weeks of life and usually not beyond three months. Frequently the baby appears normal at birth but develops purpura several hours after delivery. The course may not be benign especially when the pregnancy is associated with overt purpura. In one report consecutive infants born of a mother who had been splenectomized for idiopathic thrombocytopenic purpura succumbed on the first day of life.⁶² Megakaryocytes are increased in patients with primary thrombocytopenic purpura and when markedly decreased or absent signify the presence of an underlying condition. In patients with congenital thrombocytopenic purpura however megakaryocytes may be normal in number or frequently depressed. The persistence of an megakaryocytic condition beyond the first months of life carries a poor prognosis.

Treatment In the seriously ill infant ACTH is administered intramuscularly in the first two days in a dosage of 25 mg daily in four divided doses and for the next two days with half the amount. For the remainder of the week prednisone is substituted. It is given orally in a dosage of 10 to 20 mg daily in four divided doses. The dosage is reduced to half the amount in the second week and tapered off gradually in the third week. The maintenance of ACTH and steroid

therapy beyond these periods depend upon the severity of the disease. As in patients with idiopathic thrombocytopenic purpura individual adjustment of dosage is necessary. Although the mode of action is unknown hemorrhage is usually controlled by these agents regardless of platelet response. With severe bleeding or purpura transfusions of fresh blood are advisable.

Thrombocytopenia Induced by Drugs

Thrombocytopenia induced by drugs may be either amegakaryocytic or megakaryocytic.⁹⁰ In the former group are the myelosuppressive chemical and physical agents such as x-ray radiation which involves the other bone marrow elements as well as the platelets to produce hypoplastic and aplastic anemia. A variety of drugs have been incriminated as being among those producing thrombocytopenia. These include among others Mesantoin, thyroid depressing drugs, sulfonamides, benzol, arsenic, and chloramphenicol. Here the effect on the bone marrow is due either to individual idiosyncrasy or to overdosage and emphasizes the need for routine blood studies as a check on toxicity. Drugs used currently in the treatment of patients with leukemia, Hodgkin's disease, and other disorders of the lymphoma group are capable of producing thrombocytopenia by megakaryocytic aplasia.

Megakaryocytic thrombocytopenia may result from sensitization produced in the patient by previous administration of the drug, as in the case of Sedormid, quinine, and quinidine. It has been shown that the addition of Sedormid to platelets suspended in the serum of a sensitized patient causes the agglutination without complement and lysis with it. Patients who have recovered from purpura caused by Sedormid possess an antiplatelet antibody in the circulating blood which is capable of destroying platelets. The fact that normal serum had no such effect indicates that the abnormality in the blood of such a person lies in the serum and not in the platelets.

In patients with thrombocytopenic purpura due to Sedormid and probably other drugs the antibody acts on megakaryocytes, platelets, and capillary endothelium. In those with nonthrombocytopenic purpura the platelets for some unknown reason escape injury. The drug presumably combines with vascular endothelium, rendered antigenic and therefore capable of uniting with antibody. Thrombocytopenia due to quinine presents a similar mechanism—namely, an antibody combined with antigen consisting of the drug and platelets.⁹¹

Aplastic Anemia With Onset as Congenital Thrombocytopenic Purpura

A newborn infant was recently observed who had what appeared to be congenital thrombocytopenic purpura. After the first weeks of life, however, anemia and subsequently granulocytopenia became associated with the depletion of platelets. Although a normal bone marrow was present at the end of the first month, by the seventh month the blood, bone marrow, and clinical findings were consistent with aplastic anemia. Although this sequence so early in life is exceptional, it is significant nevertheless that congenital thrombocytopenia usually benign may represent the initial stage of aplastic anemia.

Chronic Hypoplastic Thrombocytopenia With Depression of Megakaryocytes

Chronic hypoplastic thrombocytopenia with depression of megakaryocytes is occasionally observed in pediatric practice in patients without congenital anomalies and consists of persistent thrombocytopenia with a depletion of megakaryocytes and periodic attacks of epistaxis, purpura or both. There is no involvement of red or white cell elements nor any coagulation defect. These patients must be observed for the possible ultimate development of the total picture of aplastic anemia or for evidence of secondary thrombocytopenia due to causes such as infection, drugs and leukemia or other infiltrative disorders.

For treatment of this condition successive monthly courses of prednisone or substitutes are administered especially if bleeding or purpura is excessive. If there is difficulty in maintaining platelet levels to prevent bleeding maintenance therapy may be attempted on a dosage of 2.5 to 15 mg daily. Continuous therapy even with minimal dosage necessitates alertness for the development of hyperadrenocorticism. When the administration of steroids has proved ineffective in patients with severe and recurrent nosebleeds large doses of ascorbic acid (1,000 mg daily) have occasionally resulted in improvement. The value of removing the spleen is debatable especially since this organ is not enlarged.

Congenital Hypoplastic Thrombocytopenia (Primary Amegakaryocytic Thrombocytopenia)

Rarely there appears to be a congenital absence or a marked reduction in the number of megakaryocytes without a reduction in the other elements of the bone marrow, often coexisting with multiple deformities.⁸ Among the first of these cases to be described was that reported by Greenwald and Sherman¹³ in which defective formation of megakaryocytes was associated with anomalies of the heart and thymus gland.

In another patient first observed at 3 months of age thrombocytopenic purpura was associated with congenital dislocation of the hip and bilateral absence of the radius.¹⁰ After mild improvement with ACTH and cortisone therapy splenectomy was performed at 10 months of age. Bleeding stopped, the number of platelets increased and one year later the platelets numbered 350,000 per cubic millimeter. In a similar case⁹ of primary aplasia of the megakaryocytes and absence of both radius, splenectomy resulted in a transitory increase in the number of platelets. Improvement was not maintained and the infant succumbed with cerebral hemorrhage. Similar congenital abnormalities with bleeding manifestations from birth based on amegakaryocytic bone marrow may present evidence of a leukemoid reaction.

The majority of patients succumb in the first weeks and months of life despite transfusions and splenectomy. From a broad point of view these cases of congenital hypoplastic thrombocytopenia with skeletal and cardiac anomalies may be classified as a neonatal equivalent of the Fanconi syndrome. Clinical and hematologic features do not ordinarily appear in patients with the Fanconi syndrome until later childhood.

Thrombotic (Thrombohemolytic) Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura is an acute febrile disease characterized by thrombocytopenia, purpura, severe hemolytic anemia, and transitory focal neurologic signs and symptoms. The varied clinical features are due to widespread intracapillary and intraarteriolar thrombi affecting most frequently the brain, kidneys, heart, and spleen. The occlusions are due not to agglutinated platelets alone but also to the vascular wall involvement with conspicuous proliferation. The possibility of thrombotic thrombocytopenic purpura should be considered whenever an unusually severe or fulminating purpura associated with thrombopenia is encountered.¹⁰

Pathogenesis It has been postulated that thrombotic thrombocytopenic purpura represents an immunohematologic disorder in which the autoimmune process is set in motion against the red cell, platelets, megakaryocyte, and vessel wall.⁸ The association with marked eosinophilia in the occasional patient¹² tends further to support this concept. The hypersensitive basis of the histologic picture is analogous to other collagen diseases, particularly periarteritis nodosa and lupus erythematosus, and to the necrotizing vascular lesion produced in experimental animals¹ by a variety of agents such as foreign proteins, sulfonamides, and iodine.

Clinical Course and Laboratory Findings The course is usually rapidly progressive, terminating fatally within a few days to several weeks from the onset. In a few patients the illness is more prolonged and of a relapsing nature. Petechiae and ecchymoses are few or numerous; enlargement of the spleen and liver occurs in many of the patients. The blood shows the evidence of a hemolytic anemia with jaundice in one half of the patients. Anemia, reticulocytosis, microspherocytosis, leukocytosis, thrombocytopenia, and erythroid hyperplasia of the bone marrow are present. The Coombs test is ordinarily negative, occasionally positive.¹⁰

Treatment Splenectomy, ACTH, cortisone, and antibiotics are of transitory value. Blood and platelet transfusions are supportive only. Anticoagulants such as heparin are without effect since the occlusions are caused by a primary pathologic reaction of the vascular wall to a damaging agent and in the absence of fibrin in the thrombi.¹⁰

Eczema, Thrombocytopenia, and Anemia (Aldrich's Syndrome)

The combination of chronic eczema complicated by thrombocytopenic purpura and chronic suppurative otitis media has been the subject of several reports.^{6, 11, 110} Bloody diarrhea, recurrent infection, anemia, epistaxis, and a fatal outcome are additional features. Persistent thrombocytopenia despite normal numbers and morphology of megakaryocytes has been noted.⁷ The pattern is that of a sex-linked recessive disease with transmission by unaffected females to male members.

Milk allergy was strongly suspected as a major factor in producing the eczema in one series.¹¹ An impressive feature common to the children in the latter group was the rapidity with which they developed fulminating infections after splenectomy.⁶ The outcome is fatal despite ACTH and steroid therapy. Although there

is a clinical resemblance to Letterer-Siwe disease the genetic pattern and combination of symptoms differentiate this condition from reticuloendotheliosis and other conditions in which either eczema or purpura is present. No clear evidence is available demonstrating a deficiency of an immune response to most antigens. Normal or slightly elevated gamma globulin levels were noted in one series.

Thrombocytopenia Following Transfusions

A tendency to bleed based on platelet depletion has been described in patients receiving large amounts of compatible banked blood.⁹ Several reasons have been cited for thrombocytopenia resulting from transfusions. When bleeding is excessive the patient loses both platelets and substances required in the coagulation process. In blood collected by ordinary blood bank procedures the agglutination and destruction of platelets are considerable so that the donor contributes inadequate amounts of viable platelets during transfusion. No significant thrombocytopenia results in patients receiving small amounts of blood over a period of days or weeks.

Another factor contributing to thrombocytopenia is the possible depressive effect of transfusions on platelet formation as is known to occur with erythropoiesis. A factor which reduces platelets has also been described in normal plasma which acting alone or with the recipient's diluted platelets operates to produce thrombocytopenia.¹⁰ A combination of these factors has been shown to reduce the number of platelets in infants with erythroblastosis receiving replacement transfusions although overt bleeding is rare.⁶

Hemolytic Anemia, Thrombocytopenic Purpura, and Renal Disease (Hemolytic Uremic Syndrome)

Hemolytic uremic syndrome¹¹ consists of hemolytic anemia, thrombocytopenia and renal disease clinically resembling acute glomerulonephritis with hematuria and proteinuria. The red cells in the hemolytic episodes are characteristically distorted irregularly contracted and fragmented but they return to normal during remissions.⁸ Spherocytes have also been noted in small numbers. Splenectomy and cortisone have been without value. The only effective treatment is blood transfusion which may have to be repeated at frequent intervals because of the rapid destruction of transfused cells. Thrombocytopenia necessitates the use of fresh blood.⁷ This syndrome is separated from autoimmune hemolytic disease by the negative Coombs test and the unusual red cell morphology. In many aspects it resembles a form of hypersensitivity and has been regarded as related to thrombotic thrombocytopenic purpura. This illness may be rapidly fatal¹² or be marked by recurrences,⁸ and the patient may recover spontaneously after a single episode. In the group of patients who recover spontaneously renal damage did not persist.

Cyclic Thrombocytopenic Purpura Related to the Menstrual Cycle

A physiologic decrease in the number of platelets occurs in the two weeks before menstruation in the majority of women reaching the lowest levels during

the first day of menstruation Easy bruising is a reflection of this alteration A gradual increase in the number of platelets takes place during the following two weeks ² Intermittent thrombocytopenic purpura has been described which was confined to the menstrual period and was followed by a spontaneous remission ³ In patients with idiopathic thrombocytopenic purpura the platelet count may be lowest during menstruation and highest during ovulation ⁴ Severe menorrhagia may be an indication of this change and sometimes the sole clinical manifestation of this disease

Hemangioma and Thrombopenia

Thrombocytopenic purpura has been reported in association with hemangioma in infancy ¹ A hemorrhagic tendency and bleeding into the hemangiomatous site may occur The lesions may be so extensive as to result in a fatal outcome as thrombopenia develops ² The thrombopenia hemangioma syndrome shows an increased number of young and agranular megakaryocytes in the bone marrow The occurrence of platelet thromboses within the vessels of the hemangioma conceivably results in their sequestration in the tumor with peripheral depletion of platelets ³ Elimination of the hemangioma results in return of the platelet count to normal whereas splenectomy is without effect on the blood dyscrasia ⁴ ⁵ Multiple hemangiomas and thrombocytopenia have also been reported Surgical excision resulted in severe bleeding ⁶

NONTHROMBOCYTOPENIC PURPURAS

Allergic Purpura (Anaphylactoid Purpura) ***Henoch Schönlein Purpura)***

Allergic purpura refers to a type of nonthrombocytopenic purpura accompanied by a pleomorphic urticaria like and purpuric type of cutaneous eruption in combination with gastrointestinal symptoms (Henoch's purpura) painful swelling of the joints (Schönlein's purpura rheumatica) and a tendency toward renal involvement These features are grouped together as the Henoch Schönlein syndrome with the realization that these symptom complexes can occur individually in combination or in sequence

Etiology and Pathogenesis The relationship of the purpuric state to allergy is suggested by the symptoms of urticaria diffuse erythema and subcutaneous and submucous extravasations of blood and lymph Although hypersensitivity is generally considered to underlie the Henoch Schönlein syndrome a definite allergen is only rarely identified Food and less frequently bacterial infection and drugs including antibiotics are suspected as the exciting factors A large variety of foods have been chiefly implicated—eggs milk chocolate wheat nuts and beans and to a lesser extent fish pork, lamb chicken and a variety of fruits In one patient an insect bite led to joint swelling blood tinged stools and hematuria ¹ Acute vascular purpura has also been reported following immunization with Asiatic influenza vaccine Although a relationship is suggested clinically skin tests are generally negative and more is gained by eliminating the suspected foods from the diet and observing for the subsidence of symptoms

is a clinical resemblance to Letterer-Siwe disease, the genetic pattern and combination of symptoms differentiate this condition from reticuloendotheliosis and other conditions in which either eczema or purpura is present. No clear evidence is available demonstrating a deficiency of an immune response to most antigens. Normal or slightly elevated gamma globulin levels were noted in one series.

Thrombocytopenia Following Transfusions

A tendency to bleed based on platelet depletion has been described in patients receiving large amounts of compatible banked blood.¹⁸ Several reasons have been cited for thrombocytopenia resulting from transfusions. When bleeding is excessive the patient loses both platelets and substances required in the coagulation process. In blood collected by ordinary blood bank procedures the agglutination and destruction of platelets are considerable so that the donor contributes inadequate amounts of viable platelets during transfusion. No significant thrombocytopenia results in patients receiving small amounts of blood over a period of days or weeks.

Another factor contributing to thrombocytopenia is the possible depressive effect of transfusions on platelet formation as is known to occur with erythropoiesis. A factor which reduces platelets has also been described in normal plasma which acting alone or with the recipient's diluted platelets operates to produce thrombocytopenia.¹⁹ A combination of these factors has been shown to reduce the number of platelets in infants with erythroblastosis receiving replacement transfusions although overt bleeding is rare.²⁰

Hemolytic Anemia, Thrombocytopenic Purpura, and Renal Disease (Hemolytic Uremic Syndrome)

Hemolytic uremic syndrome²¹ consists of hemolytic anemia, thrombocytopenia and renal disease clinically resembling acute glomerulonephritis with hematuria and proteinuria. The red cells in the hemolytic episodes are characteristically distorted, irregularly contracted and fragmented but they return to normal during remissions.²² Spherocytes have also been noted in small numbers. Splenectomy and cortisone have been without value. The only effective treatment is blood transfusion which may have to be repeated at frequent intervals because of the rapid destruction of transfused cells. Thrombocytopenia necessitates the use of fresh blood. This syndrome is separated from autoimmune hemolytic disease by the negative Coombs test and the unusual red cell morphology. In many aspects it resembles a form of "hypersensitivity" and has been regarded as related to thrombotic thrombocytopenic purpura. This illness may be rapidly fatal²³ or be marked by recurrences,²⁴ and the patient may recover spontaneously after a single episode. In the group of patients who recover spontaneously renal damage did not persist.

Cyclic Thrombocytopenic Purpura Related to the Menstrual Cycle

A physiologic decrease in the number of platelets occurs in the two weeks before menstruation in the majority of women reaching the lowest levels during

An infectious agent (a drug or toxin for example) may supposedly form a complex with capillary endothelium or platelets along the lines suggested by Ackroyd with Sedormid therapy thus initiating active immunization. As further confirmation of the concept of Henoch-Schönlein disease as a hyperimmune disease was the application of a precipitin test using a preparation of the aorta of newborn infants. A positive precipitin test with decalcified serum was obtained in six out of eight patients with allergic purpura and three out of four patients with periarteritis nodosa.²¹

Clinical Features Henoch-Schönlein purpura occurs more commonly in males than in females is slightly more frequent in children and adolescents and has a peak incidence in children from 4 to 15 years of age. The average duration of the disease is about four weeks. The disease is marked by a tendency toward recurrences. In a series of 131 pediatric patients with allergic purpura Allen and associates¹⁴ reported an age range of 3 months to 16 years with a median of 4 years. Three-fourths of these patients were under 7 years of age and about one-third were either 2 or 3 years old. Within six weeks of the onset of the disease 40 per cent of these patients had one or more recurrences after a period of well-being. Recurrences were more common in patients over 2 years of age than in younger patients. The occurrence of the disease in several siblings in one family is rare but it has been reported.⁹ The disease is ushered in by headaches, anorexia and abdominal pain. Fever is rarely over 101° F. The onset is variable and early in the course symptoms from the skin, gastrointestinal tract or joints or any combination may predominate. Localized edema occurs on the backs of the hands and around the eyes, face, scalp and lips. Subconjunctival hemorrhage may be present. Although skin lesions usually occur initially, colicky abdominal pain and gastrointestinal bleeding or joint symptoms may precede any type of skin rash for prolonged periods. Younger patients tend to show more frequent edema of the soft tissue of the scalp, face, eyes, hands and feet and older children are more prone to gastrointestinal and renal manifestations.⁴

Skin The specific skin lesion progresses from recurrent crops of urticarial lesions to maculopapules which are at first pink and then red. Petechial hemorrhages occur in a zone of erythema with new lesions appearing every few days. Ecchymoses, urticarial wheals and edema are common. The lesions tend to spread forming larger patches, often with a hemorrhagic component as a later development. The most commonly affected sites are the buttocks, lower back, extensor surfaces of the elbows and arms and the back of the lower leg, ankle and foot. Rash on the calves and thighs has been prominent in some patients. A petechial and purpuric eruption simulating the embolic phenomena of meningococcal sepsis may occur. Epistaxis and bleeding from the gums are rare. Histologic changes are restricted to the small vessels of the corium which are surrounded by an acute inflammatory exudate.² Tissue eosinophilia suggests a local hypersensitive reaction.

Abdominal Symptoms Abdominal pain frequently referred to the umbilical region is common. Recurrent colicky pain is the most striking symptom of the disease. The attacks are associated with vomiting, often with melena consisting of the passage of blood and mucus. The lower ileum is usually involved.

The high incidence of preceding upper respiratory infections often with beta hemolytic streptococci and the similarity between the latent period of one to three weeks until the appearance of the hemorrhagic manifestations have suggested both a close association between Henoch Schonlein purpura and acute nephritis and the hyperimmune nature of both diseases. On the other hand Bywaters and co-workers¹ found in a series of sixty four cases that although Henoch Schonlein purpura was preceded by an upper respiratory infection in 72 per cent of their patients group A beta hemolytic streptococci were isolated in only one fourth of the patients investigated. This evidence was intermediate between that found in patients with rheumatic fever and in a control series of children admitted for nonrheumatic carditis. Thus there is no clear evidence that this type of specific infection is an invariable causative factor. It may well be that the increasing use of the sulfonamide drugs and antibiotics in connection with the treatment of acute upper respiratory infections may be responsible for the apparent increasing incidence of the Henoch Schonlein syndrome.²⁰

A disturbance of the vascular endothelium resulting in increased capillary fragility and permeability is a basic factor in the symptomatology. Osler²¹ was among the first to call attention to the association between arthritis and intestinal symptoms and the erythema group of skin diseases and to emphasize the renal complication. The erythematous exanthem of the Henoch Schonlein syndrome has been differentiated from true purpura in which the surrounding skin is normal.² However the two conditions are not always separable and true purpura in the sense of this definition is often observed in children in conjunction with other features of Henoch Schonlein purpura.

The Henoch Schonlein syndrome has been linked²² with acute nephritis, rheumatic fever and polyarteritis nodosa and other collagen diseases. They have overlapping clinical characteristics and a similar pathogenesis based on an antigen antibody reaction involving the endothelium of blood vessels. Henoch Schonlein purpura can be classified as an immunovascular disorder resulting in a generalized blood vessel disturbance, an acute perivascularitis or angitis affecting the skin, joints, intestinal tract and renal glomeruli.¹⁷ Kreidberg and associates postulated a latent period between the remission of a preceding infection and the appearance of purpura during which an immunologic mechanism develops. The resulting antigen antibody reaction at the vascular level would explain the generalized vascular damage. The histopathologic features of acute necrotic involvement of vessel walls and hemorrhage which are prime features have led to the designation of this disease as allergic angitis.¹⁰⁹ The pathologic lesion in skin and kidney biopsies consists of fibrinoid thrombi in capillaries and an associated endothelial and perivascular reaction.⁹⁸

An antiserum made experimentally from vascular endothelium has been found to produce diffuse hemorrhagic purpura in the skin and internal organs. Following the direction of previous Japanese investigators Clark and Jacoby using suspensions of dog vascular endothelium from the aorta and vena cava produced a rabbit antiserum which when injected in dogs produced a generalized nonthrombocytopenic hemorrhagic purpura. The resulting lesions corresponded to the diffuse vasculitis involving small blood vessels with perivascular collections of polymorphonuclear cells, lymphocytes and macrophages observed in Henoch Schonlein disease changes which provide a basis for the increased permeability of small blood vessels.

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although both the stomach and jejunum may be affected. Colic when severe may suggest intestinal obstruction intussusception and acute appendicitis. These symptoms may precede or accompany the purpuric rash. Perforation is a rare occurrence.

Laparotomy is occasionally undertaken to eliminate these possibilities although if operation no remedial surgery is usually required. Exception is taken in the case of the patient with intussusception a rare complication of Henoch-Schönlein purpura in which operation is urgently indicated.⁶⁴ The abdominal symptoms are related to edema or extravasations of blood or serosanguinous fluid into the wall of the intestine. Despite the fact that the alimentary lesions have been compared to the skin purpura they have also been interpreted as local vasospasms secondary to damage to the arterioles. With typical abdominal symptoms surgical intervention should not be undertaken without questioning as to previous attacks of purpura. The tip of the spleen is occasionally palpable.

Joint Involvement Joint symptoms vary from transient puffiness of a single joint to recurrent warm and painful swelling of many joints often associated with a limp. The knees and ankles are most commonly affected. The swelling is due to periarthral involvement rather than intra-articular bleeding or effusion. There is no permanent damage to the joints.

Renal Complications Renal involvement has been estimated to occur in as high as one half of the children with Henoch-Schönlein purpura⁶⁷ and constitutes by far the most serious complication of the disease. Glomerulonephritis is manifested by gross and microscopic hematuria, cylindruria and proteinuria. Although the majority of children make a complete recovery some may develop a latent chronic nephritis and eventual renal failure.^{68,69} The prognosis must be guarded in those with prolonged hematuria.

The pathology of the kidneys in allergic purpura has been subject to close analysis and varied interpretation. Renal biopsy has made it possible to correlate the clinical and laboratory findings with the actual pathologic status of the kidney.^{70,71,72} It is realized that the severity and chronicity of the glomerulonephritis vary from case to case in this disease. According to Vernier and associates⁷³ the acute glomerular lesion is characterized by segmental glomerular proliferation and occlusion of capillaries by Schiff positive fibrinoid material with organized hyaline material or segmental scars in the older lesions. These authors regard the lesion not as similar to acute glomerular nephritis but rather as part of a diffuse vascular disease with lesions resembling those seen in patients with disseminated lupus erythematosus. Bergstrand and associates⁷⁴ interpret the renal lesions as remnants of an inflammatory process similar to glomerulonephritis with the histologic changes favoring a good prognosis even in patients with persistent hematuria.

Neurologic Complications Serious neurologic complications are rare. In two children the exanthema, intestinal colic, vomiting, arthralgia and nephritis were accompanied by subarachnoid hemorrhage which was fatal in one patient.⁷ In another patient swelling of an ankle joint preceded the purpuric rash and was followed by colic and fatal cerebral hemorrhage.

Laboratory Findings The bleeding time, coagulation studies and clot retraction are normal. Platelets are normal in number. Anemia and leukocytosis occur only in patients with severe blood loss and eosinophilia is rare. The tourniquet test is positive in only 25 per cent of patients.

Diagnosis The typical purpuric erythematous eruption, colic and articular and periarticular swelling in a patient with a normal platelet count differentiates the Henoch-Schönlein syndrome from the thrombocytopenic purpuras. The normal coagulation studies serve further to separate this condition from established bleeding disorders.

Course and Prognosis The disease is variable in its manifestations and duration. In the majority of patients there is only a single acute episode which clears up spontaneously in a month. Recurrences are common. In its mildest form the disease runs its course in a few days with transient purpura on the extremities and lower trunk and fleeting abdominal or joint pain or both.

At the other extreme are the serious and fatal forms often with an insidious onset with progressive purpura, severe arthralgia, gastrointestinal hemorrhages, abdominal pain, hypertension and kidney and cerebral hemorrhage. In patients with recurrent abdominal pain there is constant concern over the need for surgical intervention.

A chronic course with exacerbations over a period of years is not uncommon. In some patients successive seasonal attacks often occur in the spring with complete remissions in the intervening months. Rarely the disease progresses with the eventual clinical picture of rheumatoid arthritis and transient purpura. The prognosis for the outcome of a single attack is good and ultimate recovery, even with repeated attacks, is generally good.

Chronic renal disease constitutes the most serious aspect and the persistence of proteinuria and red cells in the urine necessitates close supervision. Prognosis is especially guarded when there is nervous system involvement or when abdominal symptoms are sufficiently urgent to require surgical exploration.

Treatment The treatment is essentially symptomatic. Bed rest is required during an attack. In cases which appear to be related to upper respiratory infections, either acute or chronic in nature, foci of infection should be investigated. Only when pathogenic organisms are definitely isolated should antibacterial therapy be instituted. Antibiotics may be of value although infrequently in our experience when given they should be used with discrimination and caution to avoid provoking or aggravating already existing purpura. Occasionally in patients with Henoch-Schönlein purpura associated with hemolytic streptococci tonsillectomy has been effective in preventing recurrences.

Antihistamine drugs, vitamins C and K, rutin and hesperidin have been of questionable value. The most favorable results have been obtained by the detection of an offending food and its elimination from the diet. Suspected foods should therefore be systematically investigated for their possible role in etiology, although this is usually not too successful. Chocolate, nuts, fish, tomatoes and other foods mentioned previously should be investigated for their effect on the occurrence of attacks of purpura.

ACTH and the adrenocortical steroids are commonly employed but with only

fair results. It might be expected that the allergic type of hypersensitivity as exemplified by Henoch-Schönlein purpura should respond because of the beneficial effect of the steroids in other members of the collagen group of diseases to which it is related. Although these drugs do not prevent relapses nor modify the course of nephritis, they are worthy of trial. In patient with moderately severe allergic purpura, steroids (prednisone or substitutes) are given orally according to age 30 to 60 mg daily in divided doses for brief periods of time. Thus treatment should be supervised especially for the effect on blood pressure elevation and aggravation of abdominal signs. In patients with severe and uncontrollable disease, ACTH or hydrocortisone is given intravenously by drip over an eight hour period in a dosage of 25 mg and 100 mg respectively. ACTH is given intramuscularly when oral administration of steroids has been ineffective.

In the series of Allen and associates⁴¹ corticosteroids were of no value in the management of the skin manifestations or renal involvement. They were found to be useful in arthralgia, soft tissue swelling, and scalp edema. These authors emphasize the need for corticosteroids in sufficient dosage for the relief of abdominal pain. The steroids did not, however, alter the frequency of recurrences nor the duration of illness.

As has already been mentioned, clinical signs indicating abdominal involvement usually do not necessitate surgical intervention. An exception is made for the patient with severe colic suggesting intussusception. The latter has been ascribed to intramural hematomas of the intestinal tract serving as a leading point for peristaltic invagination.⁴² Reduction or resection of the gangrenous segment is usually successful.

Congenital Vascular Defects

Pseudohemophilia: Pseudohemophilia, known as the von Willebrand syndrome, has been described in connection with coagulation disorders (see Chapter 26). It was shown that this syndrome could be classified into two distinct groups, both possessing a capillary defect with prolonged bleeding time. One group was demonstrated to be associated with marked deficiency in antihemophilic globulin (pseudohemophilia B); in the other the abnormality was confined to the vascular defect (pseudohemophilia A) with no abnormality of coagulation.

Hereditary Hemorrhagic Telangiectasia (Rendu-Osler-Weber Disease): Rendu-Osler-Weber disease is a hereditary condition characterized by the presence of telangiectases, either localized or generalized, and by a tendency to bleed from these lesions. It is transmitted as a simple dominant affecting both sexes, although a generation may be skipped. The individual lesions are slightly raised, are 1 to 4 mm in size, have a bright red and violaceous appearance, and consist of localized dilatations of capillaries and venules in the skin and mucous membrane. Because of their exposed nature, they give rise to profuse hemorrhage, either spontaneously or as a result of trauma. The onset is typically in childhood with epistaxis due to a localized lesion in the nasal mucous membrane. The telangiectases increase in number and become widespread in adult life.⁴³

Recurrent and severe epistaxis in childhood and in older persons justifies nasopharyngoscopic examination for possible demonstration of telangiectases. The

lesions are located most commonly in the nasal mucous membrane and less often on the palate tongue conjunctivae lips scalp ears and the face and under the fingernails Visceral lesions account for gastrointestinal¹⁰³ genitourinary and pulmonary bleeding and less often for bleeding in other organs In patients with pseudohemophilia in whom nosebleed is also severe the bleeding time is prolonged in contrast to normal findings in patients with hereditary telangiectases Borderline cases occur¹⁰⁴

Therapy consists of protection against local trauma and topical and hemostatic agents including thermocautery transfusions and iron for severe hypochromic anemia when the loss of blood is severe The disease is rarely fatal since patients rarely die from hemorrhage

Ehlers Danlos Syndrome The Ehlers Danlos syndrome is due to a constitutional and congenital dysplasia of the mesenchyme and comprises the following clinical features friability of the skin (dermatorrhexia) and of the blood vessels overextensibility of the joints (arthrochaliasis) hyperelasticity of the skin pseudotumors and freely movable subcutaneous nodules^{61,96} These pea sized nodules consist of subcutaneous fat With the onset of walking injuries sustained by the infant result in gaping incised wounds with bleeding due to tearing of the adjacent blood vessels The pathologic background is represented by scanty subcutaneous tissue thinning of collagenous fibers and an increase of the elastic tissue in the corium which is arranged in irregular and coarse bundles With the reduced subcutaneous tissue and friability of the skin the blood vessels are susceptible to trauma There is no bleeding tendency Bleeding and coagulation time are normal There is no epistaxis oozing from mucous surfaces nor undue bleeding from wounds or tooth extraction This syndrome is regarded as a hereditary disease with dominant transmission occurring in both sexes but not in all patients is there a familial incidence⁶¹



Fig 47 Ehlers Danlos syndrome Hyperlaxity of the joints is especially prominent in the fingers (From Smith C H Dermatorrhexis [Ehlers-Danlos Syndrome] J Pediat 14 63., 1939)



Fig. 48



Fig. 49

Fig. 48 Loose and hyperelastic skin stretched beyond normal limits returns to normal position when released (From Smith C H : *Dermatorrhexis* [Ehlers Danlos Syndrome] *J Pediat* 14 632 1939)

Fig. 49 High power photomicrograph of skin ($\times 260$) Note that the malpighian layer of the epidermis is reduced in cellular content The dense reticulum of coarse elastic elements in the corium and the diminution of collagen fibers are important factors in the production of the gaping incised wounds characteristic of the syndrome (From Smith C H : *Dermatorrhexis* [Ehlers Danlos Syndrome] *J Pediat* 14 632 1939)

There is no specific treatment that can alter the fundamental abnormalities of this condition Therapy consists in the attempt to increase the deposition of fat in the subcutaneous layer to act as a buffer against trauma

Purpura Fulminans

Purpura fulminans is a rare form of nonthrombocytopenic purpura characterized by anemia fever shock and sudden and rapidly spreading skin hemorrhages usually occurring symmetrically in the lower extremities¹⁰⁶ Marked prostration occurs almost exclusively in young children and follows an infectious disease The course is usually rapidly fatal within a few days Of the diseases of childhood purpura fulminans follows scarlet fever most often^{107 108} but it may also be associated with varicella¹⁰⁶ and measles Food has also been incriminated as a causative agent The basic lesion consists of extensive vasculitis which leads to necrosis of tissues Because it resembles a hypersensitivity reaction it is often

regarded as a variant of Henoch Schönlein purpura although the cardinal features of the latter (mucous membrane bleeding and joint intestinal and renal symptoms) are absent. Necrotizing vasculitis may be so extensive as to require amputation of the lower legs. Contributing to the clinical picture of purpura fulminans is the diminution in clotting factors such as labile factor (factor V)³ and fibrinogen.¹⁸ Rarely the platelet count is decreased.¹⁹

Treatment ACTH, the adrenocortical steroids, intravenous fluids and antibiotics should be given. Transfusions of fresh blood and plasma are especially useful when a clotting factor is absent.

Waterhouse Friderichsen Syndrome

Waterhouse Friderichsen syndrome is a rapidly fatal form of severe purpura associated with adrenal hemorrhage during the course of meningococcemia or other severe septicemias. Fever, shock, prostration, vascular collapse and widespread hemorrhages are prominent features. The disease usually affects infants from 2 to 15 months of age and in 90 per cent of all cases it occurs in children under 9 years old. ACTH and adrenocortical steroids are used in treatment and have occasionally been helpful.⁶¹

Idiopathic Pulmonary Hemosiderosis

Idiopathic pulmonary hemosiderosis is a disease occurring most frequently in childhood and in young adults and is characterized by dyspnea, cyanosis, fever, cough, blood streaked sputum and pallor with laboratory evidence of iron deficiency anemia. These features are associated with the basic pathologic process in which blood is extravasated in the pulmonary alveoli by repeated hemorrhages.

Clinical Course The diagnosis of idiopathic pulmonary hemosiderosis should be considered in patients with severe cough, dyspnea, dullness, rales, bronchial breathing and anemia. The course is one of remissions and exacerbations with intermittent hemoptysis and hematemesis. Acute episodes last two to ten days but occasionally persist for several weeks.^{10,4, 9,24, 55, 106, 111} Mild jaundice appears after a few days. Occasionally the disease occurs with a rapidly fatal outcome without recurrent manifestations.⁶⁴ Physical examination during an acute attack may not be striking. The roentgenogram of the chest shows a variety of findings: diffuse homogeneous opacities, bilateral peripheral patchy infiltration or diffuse pulmonary infiltrations with increase in bronchovascular markings extending from the hilar region. Clearing of the pulmonary fields occurs subsequently but roentgenographic examination shows a residual streaked reticular pattern or a persistent military type of infiltration radiating from the hilus. At the onset the disease resembles virus pneumonia or a severe upper respiratory infection. The most frequent peaks of incidence are at 1 to 2 years of age and at 13 to 15 years of age.⁵ An apical systolic murmur may be present, the spleen may be palpable and in children in whom the disease has become chronic the liver is enlarged. The disease must be differentiated from pulmonary hemosiderosis secondary to mitral stenosis. Readmission to the hospital is sought for respiratory distress, fever and hemoptysis.

Sputum gastric washings, and aspiration from lung puncture³⁷ reveal macrophages laden with granules of hemosiderin stained deep blue with the Prussian blue reaction. Intra alveolar pulmonary blood loss accounts for a hypochromic microcytic iron deficiency anemia with reticulocytosis. In two children aged 3 and 8 years under observation evidence of the iron deficiency anemia was further confirmed by a low serum iron content of the blood and a moderate increase in

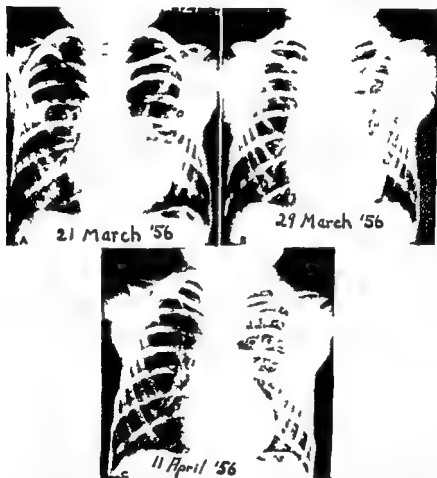


Fig. 50 Roentgenograms of a 4 year-old girl with pulmonary hemosiderosis. Note perihilar and pulmonary infiltration due to extravasation of blood into the alveoli by repeated hemorrhages. A and B. The attacks are recurrent with substantial clearing during convalescence. C.

latent iron binding capacity. Studies of the anemia and iron distribution in patients with idiopathic pulmonary hemosiderosis⁸ revealed that during the acute phase there was an excessive plasma iron turnover and an iron kinetic pattern characteristic of an iron deficiency anemia and hemorrhage and not of hemolysis. The tuberculin and Coombs tests are negative; the platelet and coagulation factors are normal. The transient jaundice and elevation of indirect reacting bilirubin in the serum following acute or subacute episodes indicate the degradation of intra alveolar blood.

Prognosis After a course of several years the disease usually ends with an acute fatal exacerbation. Terminal cardiorespiratory failure results from the effects of recurrent pulmonary bleeding. In a review by Soergel⁵⁵ the average duration was 2.9 years, the shortest 5 weeks and the longest 10 years. Of thirty-two patients, nine had died, ten were still active, five showed no progression of the disease and eight had become symptom free.

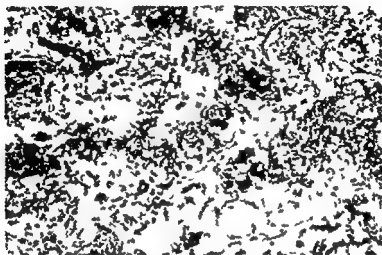


Fig. 51—Pulmonary hemosiderosis. Fibrous thickening of the alveolar septa of the lung, showing broken thin and sparse strands of elastic tissue ($\times 150$). Extensive hemorrhage into alveolar spaces, bronchioles, and small bronchi is evidenced by the presence of diffuse hemosiderosis. (Heavily stained areas are indicative of iron deposition.)

Pathology The basic pathologic process is repeated intra alveolar hemorrhages, massive in patients with acute attacks and of variable amounts in those with subacute or chronic phases. There is no hemosiderosis of other organs. The pulmonary findings in a 4-year-old child with a fourteen-month history of several exacerbations typify the usual fatal case. There was a diffuse fibrous thickening of the alveolar septa which showed broken thin and sparse strands of elastic tissue, clumps of golden pigment-laden macrophages in numerous alveolar spaces, and extensive hemorrhages into alveolar spaces, bronchioles, and small bronchi. When a special stain is employed, the pigment in the macrophages stains blue, indicating the presence of iron or evidence of old hemorrhage. Hyaline membrane formation was also found in many alveoli and in some bronchioles.

Pathogenesis The etiology of this disease has been ascribed to a primary developmental defect of the elastic fibers, with their fragmentation resulting in stasis within capillary vessels and hemorrhages. The failure to find destruction of elastic tissue in all patients led Steiner⁵⁶ to regard essential pulmonary hemosiderosis as an antigen-antibody reaction caused by a still unknown sensitizing agent inducing the production of autoantibodies. With the lungs as a shock organ, the antigen-antibody reaction produces capillary dilatation, stasis, diapedesis, thrombosis, and increased destruction of red corpuscles and deposits of hemosiderin.

Treatment For the effects of acute and chronic blood loss into the lungs almost continuous iron therapy transfusions bed rest and oxygen are required Iron from the extravasated blood in the pulmonary alveoli is available for hemoglobin formation following each episode.²¹ Antibiotics are employed for superimposed infection Based on these immunoallergic hypotheses steroids and ACTH have been advocated Favorable reports with this treatment require further evaluation because of the great variations in the frequency and severity of attacks Similarly splenectomy designed to reduce the severity of the bleeding episodes needs to be subjected to more prolonged trial

Miscellaneous Purpuric Disorders (Nonthrombocytopenic)

Vilberg and Brown²² reviewed a number of syndromes consisting of capillary disorders with and without coagulation defects Purpura simplex refers to mild skin purpura unassociated with specific blood disorders Hereditary familial purpura simplex is a condition in which spontaneous cutaneous purpura occurs on a transmissible basis involving several generations¹ The bleeding time is normal the tourniquet test is positive This type of purpura occurring in association with congenital ptosis of the eyelids has been described in eleven members of a family representing four generations²

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Treatment For the effects of acute and chronic blood loss into the lungs almost continuous iron therapy transfusions bed rest and oxygen are required Iron from the extravasated blood in the pulmonary alveoli is available for hemoglobin formation following each episode³¹ Antibiotics are employed for superimposed infection Based on these immunoallergic hypotheses steroids and ACTH have been advocated Favorable reports with this treatment require further evaluation because of the great variations in the frequency and severity of attacks Similarly splenectomy designed to reduce the severity of the bleeding episodes needs to be subjected to more prolonged trial

Miscellaneous Purpuric Disorders (Nonthrombocytopenic)

Valberg and Brown³² reviewed a number of syndromes consisting of capillary disorders with and without coagulation defects Purpura simplex refers to mild skin purpura unassociated with specific blood disorders Hereditary familial purpura simplex is a condition in which spontaneous cutaneous purpura occurs on a transmissible basis involving several generations¹ The bleeding time is normal the tourniquet test is positive This type of purpura occurring in association with congenital ptosis of the eyelids has been described in eleven members of a family representing four generations¹

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